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The Histologic Analysis of Mammary Tumors of Mice. I. Scope of Investigations and General Principles of Analysis [1, 2]

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After half a century of histologic and experimental investigation, the mammary tumors of mice defy orthodox histogenetic classification or the correlation of structure with clinical behavior. The inability to classify and name the tumors satisfactorily is important only because it implies a serious gap in understanding the biology of mammary neoplasia in mice. A survey of a varied and extensive histologic material, accumulated chiefly in the course of observations previously recorded (*1–4*), suggested that a re-evaluation of histologic structure might be useful, on condition that the studies cover the whole course of neoplasia from its earliest to its latest stages and take into account the recently acquired information about the origins of mammary tumors, their progression through various stages of development, and their responsiveness, at some stages, to extrinsic stimulation, notably by hormones. It is desirable to advance from unrewarding efforts to classify tumors to an analysis of mammary neoplasia as a dynamic process. The present paper describes the source and nature of the histologic material and discusses general principles applicable to its analysis.

Material and Methods

Most of the tumors were derived from mice previously described (*1–4*) and from similar mice examined later, and they belong to 3 main groups: 1) tumors from mice with a high incidence of spontaneous mammary tumors and believed to carry the milk agent; 2) tumors from mice with a low incidence of mammary tumors and believed free of the milk agent; 3) transplanted tumors.

Most of the tumors of the first group came from 2 series of (RIII♀ × C57BL♂)F_1 hybrids, designated RB F_1, or from the various generations of 3 strains (BR4 pink label, BR4 blue label, and BR6) inbred from tumor-bearing (C57BL♀ × RIII♂)F_1 hybrids believed to have received

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the milk agent from the male parent (*2*). Other tumors of this group derived from a series of (C57BL♀ × A♂)F_1 hybrids, designated BA F_1, injected early in life with material believed to contain the milk agent (*2*), from an inbred line of RIII, and from the early generations of inbreeding of strain MIII started by outcrossing RIII with colony-inbred M. R. C. mice.

Tumors of the second group were derived from strain BR0, inbred from a tumor-free (C57BL♀ × RIII♂)F_1 hybrid, and from (C57BL♀ × RIII♂)F_1 hybrids, designated BR F_1, one series of which, BRx F_1, had the male parent from an agent-free strain of RIII (*5*). As previously reported (*2*), some BR F_1 hybrids probably carry the milk agent, obtained from the male parent, but the majority do not. The strains BR6 and BR0 have now been inbred by brother × sister matings for over 30 generations. Strain BR6 maintains a high incidence of spontaneous mammary tumors and is believed to carry the milk agent; BR0 maintains a low and late incidence of mammary tumors and is believed to be free of the agent.

The transplanted tumors came from one or another of the groups already mentioned and were transplanted as a rule in BR F_1 mice. I am indebted to my former colleague, Dr. B. D. Pullinger, for a transplantable tumor of exceptional interest, (Yx 30), from a mouse of her agent-free line of RIII; this tumor was transplanted in males of the agent-bearing RIII strain.

Except when used for breeding to maintain the strains, the mice were force-bred. Records were kept of the reproductive histories of the mice and of the clinical course of the tumors, but they are not complete or consistent throughout the whole period of observation, which extended over nearly 10 years. With rare exceptions, mice were killed with coal gas and the tumors measured and removed immediately. Chosen tumors were fixed mostly in 10 percent formol-saline, then transferred to Gilson's Carnoy solution before being embedded in wax. Later material was fixed chiefly in Bouin's fluid. Staining methods included hemalum and eosin, Weigert's hematoxylin and van Gieson's stain, Masson's hematoxylin-erythrosin-saffron (*6*), Lendrum's phloxine-tartrazine (*7*), and the periodic acid-Schiff method.

When the bulk of the material was collected, interest centered on early "responsive" tumors, nearly all of which were preserved for histologic examination. Large tumors, which seemed as commonplace in *post-mortem* appearance as they had been in clinical behavior, were not always preserved; in particular the familiar hemorrhagic tumors are probably under-represented in this series. Tumors from the "low-incidence" strains of mice were almost always preserved. Owing to this selection of material, favoring the "early" and "unusual" forms of neoplasia, an estimate of the relative frequency of the various types of histologic structure to be described cannot be reliable, and figures here and elsewhere are given only as an indication of the range of material available for histologic examination. Omitting the transplanted tumors, the analy-

sis is based on material from 710 mice, of which 620 belonged to the "high-incidence" group and 90 to the "low-incidence" group. In the high-incidence group there were about 200 "plaques," or early stages of neoplasia, 870 fully developed tumors, and about 200 lesions in which both "plaque" and "tumor" were present in highly varied proportions. The distinction between "plaque" and "tumor" is sometimes arbitrary, as will be described elsewhere, and in the "low-incidence" group, with 112 mammary growths, the distinction cannot be made.

General Characteristics of Mammary Neoplasia

It is now widely believed that the "hyperplastic nodule" first described by Apolant (*8*) and, in more detail, by Haaland (*9*) is the starting point of most mammary tumors in the "high-incidence" strains of inbred mice. The peculiarity of the present material is the frequent manifestation of early neoplasia in the form of clinically apparent discs or plaques, measuring usually about 0.5 to 1.0 cm. in diameter and 0.1 to 0.2 cm. in thickness when first detected, which grow only during pregnancy and regress quickly after parturition (*4*). These plaques, made up characteristically of radially disposed branching tubules in symmetrical arrangement, are described in detail in another paper (*10*), where evidence is given for the belief that nodules and plaques are at corresponding stages of neoplastic development and represent alternative, not consecutive, steps in the progression of mammary neoplasia. The plaques, being of larger size and amenable to clinical examination, give exceptional opportunities for the investigation of early stages of neoplasia.

According to evidence presented elsewhere (*10*) the majority of tumors in the present series originate not in hyperplastic nodules but by progression within plaques. In other respects the tumors correspond, in general, with those described often before in mice of diverse origins. The pioneer investigators in experimental cancer research, notably Apolant (*8*), J. A. Murray (*11*), Haaland (*9*), and Bashford (*12*) gave detailed histologic descriptions and more recently Cloudman (*13*) and Dunn (*14,15*) have reviewed the histology of the tumors. Dunn's valuable review of the early observations in conjunction with her personal experience leads her to the conclusion that the tumors do not lend themselves to classification owing to the wide variability of structure, even within single growths (*14*). For some purposes a division into broad groups on the basis of predominating histologic types, as made by Dunn (*15*), is legitimate and useful. For closer histologic analysis it is necessary and practicable to distinguish various types of histologic structure within tumors. Descriptive terms previously used unsatisfactorily for classifying tumors are often useful to a greater or lesser degree for describing particular *components* of tumors, although not for the whole growth.

The most abundant tumors in the present series belong to a heterogeneous group of *varied* growths which it is unprofitable to classify further. The tumors differ widely from one another, and individual tumors usually contain several diverse constituents. The group includes Cloudman's

adenocarcinoma (*variable type*), Dunn's *Type B*, and Bonser's (*16*) *irregular tubular* and *intratubular* types. In my material irregular tubular and intratubular growths are especially conspicuous. Hemorrhage and edema, as well as milky secretion and epidermoid differentiation of minor degree, often complicate the structure of *varied* tumors. The majority of the commonplace mammary tumors belong either to this group or to another group comprising the growths described variously as *adenoma simplex* (*8*), *simple adenocarcinoma* (*13*), *Type A* (*15*), or *small tubular* (*16–18*). The term *microtubular*, slightly modified from Bonser's *small tubular*, seems most appropriate for the tumors of my own series. In some previously reported series, tumors of this group outnumber the polymorphous tumors. Microtubular growth often occurs in fairly "pure" form, especially in small tumors, but more complicated growths link the microtubular with the *varied* tumors.

Factors in the Determination of Histologic Structure

As first remarked by Haaland (*19*), the most characteristic feature of mammary adenocarcinoma in the mouse is its tubular structure. The great diversity in the histologic features is accountable in the main to variation in 3 characters, namely, 1) the architecture of the tubules; 2) the degree and direction of epithelial proliferation; and 3) the degree and type of epithelial differentiation. Secondary complications often distort the primary structure. In the analysis of histologic structure, it is necessary to consider all these factors and, additionally, the variability of structure, the independence of tumor characters, and the responsiveness of tumors to extrinsic stimuli. The following paragraphs discuss these matters separately.

Tubular Architecture

The unit of structure in the plaques from which the majority of tumors in the present series derive is a branching tubule, and at least some of the hyperplastic nodules reported by others in different strains of mice are tubular. Most, if not all, of the predictable variants of the tubular form are recognizable in mammary neoplasia. The tubules may be long or short, straight, curved, or, as in microtubular carcinoma, tightly coiled; their bore may be small—again as in microtubular carcinoma, irregular—as in Bonser's *irregular tubular* type, or large; or the tubules may be widely dilated into cysts, with or without evagination or invagination of the walls.

Murray (*11*) refers to a "tendency to organoid arrangement." Exceptionally, the arrangement of tubules in an orderly pattern dominates the tumor structure, which is unmistakably "organoid." In most carcinomas the arrangement of tubules is disorderly, but radially disposed branching structures are discernible fairly often at the growing edges.

Epithelial Proliferation

Epithelial proliferation that just keeps pace with the lengthwise growth of the tubule provides a uniformly single-layered lining. However,

the epithelial proliferation is often excessive and the lining of the tubule is, in consequence, uniformly or patchily multilayered. The excess proliferation may be slight and regular, producing, for example, a uniformly 2-layered epithelium, or it may be enormous. The overgrowth of epithelium, as first described by Murray (*11*) and mentioned more recently by Bonser (*16–18*), may extend inward or outward. Predominantly inward or centripetal growth leads to the *intratubular* type of Bonser and to the *intracanalicular* type of Cloudman. Intratubular growth encroaches on, and finally eliminates, the lumen of the tubule, which is converted into a solid cylinder with a sharp peripheral outline. When the epithelial proliferation is predominantly outward or centrifugal, the lumen of the tubule remains conspicuous and is often lined by a regular layer of epithelium outside which is a halo of irregular, proliferated epithelial cells.

Epithelial Differentiation

In an earlier general review of the histologic analysis of tumors, I gave evidence from various sources to show that the types of differentiation possible in tumors are those of which the parent tissue is capable and that the capacities of normal adult tissues for varied or divergent differentiation are far wider than commonly supposed or than revealed in health. Among them, the tumors derived from a single tissue probably can show all the types of differentiation of which the parent tissue is capable, but a particular tumor is often limited rather narrowly to one of the possible paths of differentiation, and that path may be one which the normal tissue follows only under the exceptional circumstances resulting from experimental interference or disease (*20*). A comprehensive analysis of mammary neoplasia in mice needs more systematic attention than has yet been given to the possible divergent differentiations of normal and neoplastic mammary epithelium.

In women, and somewhat less consistently or certainly in rodents, the normal, resting mammary epithelium is double-layered. Dawson (*21*) describes an outer layer of basal cells and an inner layer of lining cells. The mammary tree grows by proliferation of the basal cells which make up the solid end-bulbs at the growing tips. The solid bulbs hollow out and elongate to form new tubules with a 2-layered epithelium, the lining cells being formed from basal cells. Normal development advances, in short, by the proliferation of basal cells and the subsequent differentiation of some of them into lining cells. The lining layer seems unstable and its function is not evident; it is shed during late pregnancy and lactation when the basal layer has been differentiated into a single-layered secreting epithelium. After this differentiation, according to Dawson, the basal layer is incapable of regeneration. Although questioned by Dawson, the existence of peripheral myoepithelial cells seems to me adequately demonstrated. In normal breast, the satisfactory demonstration of myoepithelium demands special histologic methods (*22*); the difficulty of recognizing it in routine histologic material probably accounts for much skepticism about its existence.

Counterparts of the aforementioned cell types are present in mammary tumors of mice. In intratubular carcinoma there are, commonly, 2 contrasting types of cells—the one pale-staining, the other dark-staining—corresponding with basal and lining, or "luminal," cells, respectively. Sometimes the wall of a tubule is regularly 2-layered, the outer layer consisting of the paler cells and the inner layer of the darker cells. In some tubules the pale columnar cells form a continuous single layer which encloses a mass of darker cells filling the tubules; elsewhere the paler basal cells seem to proliferate to fill the tubules, or pale and dark cells are present in a confused mixture. The tubular epithelium may differentiate into either a secreting epithelium or into a keratinizing squamous epithelium. Some tubules are partially or completely filled by pale cells of a type different from the basal cells already mentioned and corresponding possibly with the "pale" epithelium seen in some mammary lesions in women (*23*). Additionally, there are at least 2 forms of centrifugal growth. In the one form, there is a distinct lumen bordered by a single layer of dark cells, surrounding which is a halo of proliferating paler epithelial cells; in the other form cells of myoid type stream peripherally from tubules or cysts lined by a single-layered epithelium of indeterminate type. In the first form the proliferating cells are apparently of "basal" type, whereas in the second form they are myoepithelial.

The various types of neoplastic mammary epithelium, including the basal, luminal, secreting, epidermoid, and myoepithelial, are not histogenetically distinct. From his examination of the glandular and keratinizing squamous components of an *adenocancroid*, now called *adenoacanthoma*, Murray (*24*) decided that " . . . the 2 differentiations . . . are inherent in cells of one type . . . ." and subsequent pathologic and experimental observations amply confirm his inference. Hamperl (*25*) believes similarly that myoepithelium is one of the possible differentiations of mammary epithelium. In general, the varied types of epithelium are different physiologic states, different "growth forms" (*11,9*), or "divergent differentiations" (*20*) of cells of a common ancestry.

Secondary Complications of Structure

Early observers made detailed studies of the secondary complications due to hemorrhage and edema in the stroma or parenchyma of mammary tumors (*8,11,14,26*). These modifications are often sufficiently pronounced to lead to fairly distinctive histologic types like Apolant's *Adenoma cysticum oedematosum s. haemorrhagicum* and *Cystocarcinoma haemorrhagicum*. Gierke (*26*) devoted a paper to the *hemorrhagic mammary tumors* of mice. In the present material these complications are frequent in some degree, but extreme forms are not abundant owing, possibly, to selection of material. Edema often confuses the picture, but, contrariwise, sometimes helps the analysis by revealing separate units that ordinarily are obscured by compression.

Variability of Structure

Early transplantation experiments showed that the structure of mammary tumors of mice is "variable" as well as "varied." Bashford (*12*), while stressing the frequent constancy of structure and behavior of transplantable tumors over long periods of time, refers also to their histologic plasticity and remarks that variations in one and the same tumor strain at different times are so great that pathologists ignorant of the life history of the tumors would regard them as of different histogenesis. He also states that "Were it possible to mix together all the daughter tumours propagated in a vast number of mice from a spontaneous or mother tumour, one would again have the confused histologic picture of a parent spontaneous tumour reproduced upon a magnified scale but with a knowledge of how it had been obtained."

Bashford distinguishes between temporary and permanent changes occurring during transplantation. The 2 types of change correspond with those currently indicated by the more generalized terms *modulation* and *progression* (*27*). Modulation applies to temporary, reversible alterations in structure or behavior. Transplantation often discloses a capacity for modulation, especially during early transplanted generations when tumors may become even more polymorphous than the primary growth. Continued transplantation shows that the modifications are reversible; they do not "breed true." As Bashford notes, the development of one differentiation does not preclude the later development of another. The capacities for divergent differentiation of the parent tissues set the broad limits of modulation of the tumors derived from them, but in individual tumors the limits may be variously narrowed. The range of modulation is wide in the *varied* tumors. The range is much restricted in some other tumors of relatively "pure" type; although, as Bashford rightly says, there is no tendency for different lines of transplantable tumor to converge to a common end point, there is a tendency to attain greater stability of structure and behavior.

Progression applies to the development of a tumor by permanent, irreversible changes in structure or behavior; these changes persist or advance during continued transplantation. Haaland (*9*) recognized the possibility of successive stages of development and that ". . . the malignant transformation may take place by degrees and not necessarily in one step from the normal cell to the fully developed cancer cell. . . ." Recently a more precise conception of tumor development as a progression though a sequence of irreversible qualitative changes has been advanced (*4*, *27*, *28*). Evidence based on the present material will be given in subsequent papers to show that mammary tumors often originate by progression within plaques and that their properties can change further by progression at later stages and even after prolonged transplantation. Often, as Haaland observed, a spontaneous tumor contains histologic traces of its history, and the presence of different developmental stages contributes to the histologic complexity. Bashford's reservation that what look like different stages of development are not necessarily consecutive steps in an advancing

sequence is in harmony with conclusions reached by myself on different grounds—that a tumor does not necessarily traverse all the theoretically possible intermediate stages of development but may vault or bypass them (*4*, *27*, *28*). Moreover tumors may follow different paths to arrive at similar end points. Although microtubular carcinoma seems to be the simplest, basic type of mammary tumor, it need not be the forerunner of the more varied and complex types, and, according to my evidence, as a rule it is not.

Both modulation and progression complicate the histologic analysis of tumors. The reversibility of modulation is demonstrable by transplantation. Progression seems to involve an irrevocable choice between several available paths of development; it entails also a restriction of the capacity for modulation. If the restriction occurs early and in extreme degree it results in the uncommon but highly instructive growth of a tumor of "pure" histologic type. More commonly, the immediate result of progression is increased complexity; simplification may occur later if the changed tumor overgrows its forerunner or if it is selected out in the course of serial transplantation.

Independence of Tumor Characters

An analysis of the progression of tumors leads to a general proposition that the structure and the behavior of tumors are determined by numerous unit characters, which, within wide limits, are independently variable, capable of assortment and combination in a variety of ways, and liable to independent progression (*27*). The outcome is a diversity of patterns of structure variously combined with varied patterns of behavior. The several histologic characters of tubules can be variously combined among themselves and with biological characters, such as growth rate and responsiveness to hormones. The conspicuously organoid manner of growth, with radially disposed branching tubules, is characteristic of responsive plaques, which are conditional growths dependent on hormonal stimulation; when, unusually, the epithelium is responsive to lactogenic hormones also, it secretes milky fluid and the plaque becomes a cystadenoma. The same organoid architecture persists in some hormone-independent tumors even after transplantation; in some, the epithelium is undifferentiated and of basal type, whereas in others it is of keratinizing epidermoid type, and the tumors belong to Haaland's *molluscoidal* type. At least 4 different types of growth thus result from combination of 1 type of tubular architecture with varied hormone responsiveness and epithelial differentiation. Similarly 1 type of differentiation, for example the keratinizing squamous, can combine with varied kinds of tubular architecture to produce various types of adenoacanthoma, as well as the uncommon molluscoidal tumor.

Responsiveness to Extrinsic Factors

Of the tumors developing sparsely in low-incidence strains of mice, a high proportion belong to histologic types that are uncommon in high-

incidence strains (*15*). In my material, adenoacanthoma preponderates in the low-incidence group. Etiologic factors, it seems, are important determinants of histologic type. Furthermore, observations to be reported later show that extrinsic factors, in particular, hormones, conspicuously alter the structure of tumors that are responsive to them and that the responsiveness may persist in late stages of neoplasia and even following transplantation. Milky secretion, the consequence of responsiveness to lactogenic hormones, profoundly alters histologic structure at early and at late stages of neoplastic development. The possible importance of extrinsic factors, of which hormones are the most amenable to investigation, in the determination and modulation of histologic structure deserves close attention.

Summary and Conclusions

Useful histologic analysis of mammary neoplasia in mice is hardly possible without a working hypothesis, which this discussion of general principles is designed to supply. Three main propositions are advanced to guide investigation: 1) mammary neoplasia in mice advances by progression through successive stages; 2) etiologic factors operating at the time of initiation probably help to determine the histologic type of a tumor, and extrinsic factors acting at late as well as at early stages of development substantially modify the structure; 3) structure and behavior depend on numerous unit characters which can combine in a great variety of ways and which are, within wide limits, independently variable and liable to independent progression.

Early and late stages of neoplasia illuminate each other. Traces of all stages of development sometimes persist in late stages of tumor growth. Some tumors are composite growths resulting from the fusion of several growths originating in separate foci. The earliest recognizable "tumors" are situated variously in "nodules," in "plaques," within ducts, or in apparently unaltered breast tissue; the possibility that the different origins are correlated with different predominant types of structure needs investigation. At one stage of neoplasia in plaques, growth is notably responsive to and dependent upon hormonal stimulation; responsiveness may persist in large and even in transplanted tumors, and the histologic structure may change substantially in response to extrinsic stimuli.

The presumption made here that the structural unit of mammary tumors of mice is a tubule is serviceable in histologic analysis, but it does not imply that all tumors pass through a simple tubular stage, and it carries no implication about the point of origin of tumors. Many histologic complexities are referable to variations in the tubular architecture and in the degree and direction of epithelial growth and differentiation; these characters are independently variable and can combine in an almost infinite number of ways.

Mammary epithelium and many of the tumors derived from it have wide capacities for modulation and divergent differentiation. Tumors contain cells comparable with the "basal" cells of the normal breast;

these cells can proliferate without differentiation or they can differentiate into luminal cells, secreting cells, epidermoid cells, or myoepithelial cells. According to Hamperl (*25*) the myoepithelial cells, in turn, can differentiate into various kinds of connective-tissue cells. Divergent differentiation from a common starting point and progression of unequal degree along one or another of the available paths are sufficient to account for the diversity of epithelial types. It is not necessary or reasonable to assume that each kind of neoplastic epithelial cell must originate from parent normal cells of analogous type; it is more important to investigate the factors which determine the choice of path and the speed and extent of progression along it. Progression involves a limitation of the capacity for modulation. The broad capacities for modulation in *varied* tumors contribute to their histologic complexity. In other tumors one of the possible modulations becomes fixed by progression and the histologic structure is relatively "pure" and stable. These tumors, although uncommon, give exceptionally favorable opportunities for the study of some unit characters.

The complexities of histologic structure are referable, according to the present argument, to several factors, including the mode and circumstances of origin of the tumors, their responsiveness to external stimuli, secondary modifications of structure, and to the highly varied assortment of unit characters, which, within wide limits, are independently variable and prone to independent modulation and progression. Currently, the most promising approach to unraveling the complexities seems to be the separate study of unit characters as they reveal themselves at different stages of neoplasia, in relation to different etiologic and environmental factors, and in a variety of tumors—including especially those possibly rare tumors in which the chosen character is predominant. Subsequent publications will describe the application of these principles to various components of mammary tumors.

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The Histologic Analysis of Mammary Tumors of Mice. II. The Histology of Responsiveness and Progression. The Origins of Tumors [1,2]

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An earlier paper (*1*) described clinical observations on spontaneous mammary tumors that developed in certain hybrid mice and their inbred descendants. Many of the tumors were notably *responsive* to reproductive activity of their hosts; the tumors grew during pregnancy and regressed partially or completely after parturition. Other tumors were *unresponsive* and grew steadily from their first appearance, independently of reproductive activity. The responsive tumors often altered their behavior while under observation, losing their responsiveness unpredictably and usually abruptly as a result of an irreversible qualitative change or *progression* in the tumors. The altered tumors then grew like the *primarily unresponsive* tumors. The present paper deals with the histology of early stages of mammary neoplasia in these mice and with the histologic manifestations of responsiveness and progression.

Material and Methods

The histologic material was derived from mice enumerated in the preceding paper (*2*). Small growths were removed in attachment to overlying skin, which was stretched out and fixed to strips of cork with hedgehog quills and immersed whole in 10 percent formol-saline. On the following or a later day the growths were sliced perpendicular to the skin surface and 1 or 2 slices through the central portion were passed through Gilson's Carnoy fluid before embedding in paraffin wax. Exceptionally, growths were sectioned parallel to the skin surface. The staining methods were those previously described. Records of the clinical history of the tumor and of the reproductive history of the mouse were usually available.

General Features of Early Neoplasia

Whole mounts were not used in a search for nodular hyperplasia, which is widely accepted as a precursor of mammary tumors in inbred mice;

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its frequency in the present series therefore is unknown, but it is certainly not the conspicuous feature as described in other strains of mice with a high incidence of mammary tumors. Chance findings provide examples of the origin of tumors in nodules, in ducts, and in apparently unchanged mammary glands as described previously by others, but the characteristic manifestation of early neoplasia is a plaque or disc measuring usually about 0.5 to 1.0 cm. in diameter and 0.1 to 0.2 cm. in thickness when first recognized clinically. Smaller plaques, not recognizable during life, are found *post mortem;* they have the same form as the palpable lesions and are visible to the naked eye and larger than most hyperplastic nodules. Plaques are usually round or oval in outline and elliptical on coronal section. They often occur simultaneously in different breasts in the same animal and sometimes 2 or 3 are present in a single breast.

Histologically, plaques are usually smoothly outlined but not encapsulated and they are notably symmetrical and organoid in structure. The simplest ones comprise a central portion or "medulla" of loose, fatty connective tissue in which normal-looking tubules are scattered and a more compact "cortex" of radially disposed branching tubules (figs. 1 and 2). There are many variants of this simple arrangement but the characteristic units in all are the branching tubules, which respond conspicuously to reproductive activity. The following account of the histologic changes during pregnancy and the puerperium is applicable in essentials to all the variants, whose special features are described later; it is based on the examination of about 200 uncomplicated plaques and a similar number with varied degrees of progression to carcinoma.

Histologic Changes during Pregnancy and the Puerperium

Plaques removed from mice during late pregnancy are characterized by abundant overgrowth of the tubules in the peripheral "cortex"; in Dawson's (*3*) terminology, there is both adenosis and epitheliosis. The central area, little changed, contains well-separated tubules lined by a single layer of cuboidal or columnar epithelium (figs. 1–3). Outgrowths from the central tubules extend in a fairly straight course, with repeated branchings, to the periphery of the disc. In the cortex the arrangement of the tubules is predominantly radial. The epithelium, single-layered in the medulla, changes, often abruptly, to multilayered in the cortex, and the tubules commonly end at the periphery in pyriform expansions resembling the end-bulbs of normal mammary development (figs. 3 and 4). At the height of epithelial proliferation mitotic figures are abundant. There is considerable diversity of cell type and arrangement in the hyperplastic epithelium. The predominant cells are large, with basophilic cytoplasm and strongly staining nuclei. In addition there are at least 2 other varieties of cells—the one smaller with dense, hyperchromatic nuclei and the other larger with pale-staining cytoplasm and large, pale, vesicular nuclei (figs. 4 and 5). Small, dark-staining cells disposed concentrically sometimes form a conspicuous layer outlining the tubule (fig. 6). Occasionally all the cells of a tubule are of a large, pale type, or a

zone of pale cells is interposed between an inner and an outer layer of darker cells (fig. 7). More commonly, different kinds of cells are mixed, in varied proportions and without apparent order (figs. 4 and 5). When the growth is predominantly centripetal, filling the tubule and eliminating the lumen, the outermost cells often form a fairly regular palisaded cuboidal or columnar layer. Sometimes the tubule is filled with radially disposed cells or, rarely, with spindle- or oat-shaped cells (fig. 8) but more usually the cells are in irregular arrangement and of mixed types, and there is often a resemblance to intratubular carcinoma, with traces of whorling and cribriform structure (figs. 4 and 5). When growth is predominantly centrifugal, the lumen persists, although often reduced to a slit. The hyperplasia is often notably irregular and asymmetrical; in cross section one portion of the tubule wall is formed by a single layer of cuboidal or columnar epithelium and the remainder by a mass of hyperplastic epithelium (fig. 5). In tubules of this kind the lumen is eccentric and often crescentic; the single-layered epithelium is sometimes continuous round the lumen (fig. 5) or seems to split into 2 layers, between which the main mass of hyperplastic cells lies. A loose cellular stroma divides the plaque into lobes and lobules. It varies greatly in amount, being sometimes abundant and sometimes so sparse that contiguous tubules touch each other (figs. 3–7). It contains many capillaries but few fibers, and the plump connective-tissue cells have large, vesicular nuclei.

Epithelial proliferation reaches a peak shortly before parturition, and signs of regression are discernible about the time of parturition or within a few hours thereafter. Often at this time pale cells are abundant; it is possible, but not certain, that they are degenerating cells. The earliest sure sign of regression is degeneration of epithelial cells, with karyorrhexis and vacuolation of cytoplasm (fig. 9). Sometimes degenerating cells are scattered irregularly through the masses of hyperplastic epithelium, but as a rule the peripheral cells seem to degenerate first and at a later stage the "ballooning" of the cells makes a clear space between the stroma and a single layer of cells bordering the lumen of the shrunken tubule (fig. 10). Uncommonly, degenerated cells are shed into the lumen and the outermost layer persists as a smooth lining of a dilated tubule (fig. 11). How the debris is removed is not evident; there is no conspicuous phagocytosis. The persistent tubules have a ragged, disheveled appearance, due partly to the peripheral ballooning of degenerating cells and partly to the irregularity of the shrunken epithelium, which includes cells with small dark nuclei and others with large pale ones (figs. 10 and 12). The further course of events in those plaques which regress completely after parturition, so that they are not detectable either clinically or at *post-mortem* examination, is not known. In those plaques which persist at a greatly reduced size during intermissions of breeding the stroma undergoes sclerosis as the epithelium degenerates; collagen fibers form early and make a dense fibrous tissue containing few blood vessels (figs. 13 and 14). In some regressed plaques the peripheral sclerotic zone is extremely thin or

incomplete and in others the usual sharp outline is lacking and tongues of fibrous tissue extend irregularly into the mammary fat. In several mice the site of growths which had regressed many weeks earlier was inconspicuous and sections showed a thin plaque with irregular fibrosis and a few shrunken tubules (fig. 15). Some of these old plaques, in their clinical and histologic features provide transitions between growths that persist as distinct palpable discs after shrinkage during the puerperium and growths that disappear altogether. The clinical and *post-mortem* recognition of regressed plaques depends on the continued presence of a layer of sclerotic tissue occupying the whole or a substantial proportion of the periphery.

Regression follows a similar course in the compound plaques and other variants of the simple plaque form, which are described in a later section (figs. 16–18).

The earliest stages of recurrence of growth in plaques that regressed after an earlier pregnancy are poorly represented in the histologic material; most mice were not killed until the typical features of the pregnancy plaque, as already described, had been reconstituted. From the few examples available it seems that the first evident change, at about mid-pregnancy, is in the fibrous stroma, which loses the specific staining properties of collagen, becomes hyaline, and gives place to a cellular and vascular stroma. Contemporaneously, the tubules lengthen and branch and epithelial cells enlarge and multiply, but mitotic figures are not abundant until recurrence is well under way.

Variant Types of Plaques

Many plaques diverge widely from the simple structure already described. The variations are due in the main to diverse combinations and arrangements of the systems of branching tubules of which all plaques are composed. The variants are separable into 2 main groups, namely, those in which the plaque form is imperfect or incomplete and those in which the association of units is more complex than in simple plaques.

Imperfect and Incomplete Plaques

In some otherwise typical plaques 1 or more segments of the cortex lack hyperplastic tubules, although the plaque outline is regular and entire (figs. 19 and 20). Several "half-plaques" have the typical plaque structure, but almost exactly one half of the elliptical plaque is undeveloped (fig. 21). In other incomplete plaques less than one quarter to more than three quarters of the ellipse fails to develop (fig. 22). The incomplete end is often sharp, as if a portion of the disc had been cut away (fig. 21). One specimen contains a lesion interpreted as a small sector of a plaque; it is roughly pyramidal in shape, with the base forming a small segment of an ellipse, and is made up of branching tubules diverging from a duct (fig. 23). This probably represents the smallest subdivision of a plaque and constitutes a single unit system of tubules.

The plaque outline is lacking in imperfect plaques, which consist of unit systems of branching tubules variously disposed and aggregated but not arranged according to the typical architecture of plaques. Some are symmetrical, but spheroidal rather than ellipsoidal, and lack the central medulla and the smooth outline of plaques (figs. 24 and 25). Others consist of systems of tubules that are scattered sparsely or abundantly, but irregularly, through a field which corresponds in extent with a plaque. At one extreme, isolated units are widely scattered or even solitary (figs. 26 and 27); at the other extreme, the units combine to form lesions closely resembling typical plaques in size and arrangement. Between the extremes, the units combine to form "nodules" which correspond with various forms of "diffuse nodular hyperplasia" and "ductular hyperplasia" described by others (*4–9*). It is noteworthy that the more frequent compact or acinar type of hyperplastic nodule is not made up of unit systems of tubules like those in plaques and that some lesions of "nodule" size are histologically typical plaques (fig. 28). Although the histologic distinction between plaque and nodule is not absolute, the linking forms are uncommon. There is no evidence to show that nodules grow into plaques, which are alternative, not consecutive, manifestations of early mammary neoplasia.

Complex Plaques

The main variants are separable into a few groups for convenience of description but the divisions are not sharp.

1) Multilobular plaques.—Multilobular plaques comprise several lobes whose medullary zones communicate, each lobe resembling a simple plaque. The external plaque form is variously distorted, but the internal architecture is, in essentials, unchanged. Several noteworthy multilobular growths underlie the nipple and seem to derive from several divisions of the mammary duct. They are responsive, and both growing and regressed forms are seen (figs. 16–18); the only peculiarity is that during regression hyperplastic tubules are apt to persist at the base of the nipple.

2) Giant plaques.—Some growths of large size retain, to a remarkable extent, the organoid internal architecture of plaques and are designated *giant plaques.* Some giant plaques with a single medulla look like greatly magnified simple plaques (fig. 29). Others are multilobular (fig. 30) and variously complicated but maintain conspicuously the organoid structure, although the disc shape may be lost.

3) Irregular complex plaques.—Many plaques lack the orderly, symmetrical internal structure of simple plaques. The systems of branching tubules are variously disposed. Sometimes they are in several layers at different depths below the plaque margin, or their radial disposition is inconspicuous or lacking (fig. 31). Some complex plaques reach a large size and the distinction from giant plaque is not precise; it depends mainly on the regularity and perfection of the organoid structure, which in complex growths is often obscured. When adenosis and

epitheliosis are maximal, the tubules become tightly packed, often with apparent fusions between contiguous tubules; mitoses are numerous in the epithelium and the stroma is reduced to a minimum; the complex and confused structure is then hardly distinguishable from that of a malignant tumor and often during pregnancy the distinction cannot be made. Observations on growths removed soon after parturition, when signs of degeneration are plain although the peak structure is still discernible, show that highly complex growths are responsive and regress in nonpregnant mice. The interpretation of the structure of growths removed beyond the narrow limits of time within which a determination of responsiveness is practicable is difficult and usually indecisive. In some growths the individual tubules, although tightly packed and in confusion, seem comparable with typical plaque tubules, but in others the resemblance to intratubular carcinoma is extremely close. The interpretation is further complicated by signs of invasion of the panniculus carnosus, but since these signs are also present in some simple plaques regressing after parturition, they do not indicate independence from hormones (figs. 13 and 17).

4) Superficial plaques and epidermal reaction.—In some plaques the tubules for the most part lie parallel with each other and with the skin surface without conspicuous branching. Radial disposition of the tubules is often apparent to some degree at the poles but not elsewhere. Epitheliosis is not pronounced but the tubules are closely packed with scanty, intervening stroma (figs. 32 and 34).

The most notable of these plaques are not, like typical plaques, wholly subcutaneous but invade the dermis. Some tubules at the superficial margin turn at right angles to the bulk of their fellows and extend toward the epidermis, immediately below which they end in expansions lined by hyperplastic epithelium (fig. 34). The overlying epidermis is epilated and thickened; histologically, it contains many empty hair follicles, some of them much dilated, into which superabundant sebaceous glands open. Hyperplastic epidermis lines the mouths of the follicles and covers the intervening skin surface, forming a fully stratified squamous epithelium (fig. 33). Hyperplastic epidermis grows downward in solid cords, with or without traces of hair, or as hollow tubes. Some of the downgrowths fuse with the hyperplastic subepidermal tubules derived from the plaque; the result is a sinus, lined in its more superficial portions by stratified squamous epithelium of epidermal origin and more deeply by glandlike neoplastic mammary epithelium; the 2 types of epithelium together make a continuous lining and the line of fusion is not clear-cut (fig. 33).

The bulk of the plaque regresses after parturition like a typical responsive plaque but the epidermal changes, the sinuses, and the hyperplastic expansions of the plaque tubules below the epidermis persist; they do not advance during intermission of breeding yet they do not regress. The persistent epidermal and subepidermal changes correspond closely in extent with the maximum size observed clinically during the preceding pregnancy. Many measurements made during intermissions of breeding were recorded as of doubtful accuracy; some of them seem in-

compatible with the *post-mortem* measurements. It is now evident that the clinical measurements apply mainly to the epidermal changes, whereas the *post-mortem* figures refer to the underlying shrunken plaque. Several plaques contain foci of carcinoma, but there is no consistent relationship between carcinoma and epidermal change, which is coextensive with the maximum growth of the plaque during pregnancy (fig. 32).

The interpretation of the histologic findings is difficult and indecisive. The epidermal changes are almost certainly secondary to the underlying plaque and unrelated to foci of carcinoma. I have seen no comparable lesions in mouse skin without an underlying plaque. The most conspicuous hyperplasias of epidermis and subepidermal plaque tubules are often closely apposed as if they had induced growth in and toward one another, the culmination being fusion of the 2 tissues to form a sinus. Some epidermal lesions have no plaque tubules in apposition to them; they are attributable, perhaps, to secondary effects of dermal fibrosis rather than to a primary inductive action of plaque tubules. The proximity to epidermis seems to modify the behavior of plaque tubules. The subepidermal tubules do not regress, as does the rest of the plaque, after parturition. This seems equally true of growth within the nipple when the latter is involved in the growth of some multilobular plaques which otherwise regress in nonpregnant mice.

5) Varied differentiations of tubular epithelium.—In plaques of characteristic architecture from mice of the high-incidence strains, there is wide variation in the degree and type of epithelial proliferation. Epitheliosis is minimal in the longitudinally ordered plaques mentioned in the preceding section and adenosis predominates. Varied combinations of epitheliosis and adenosis are present in more typical plaques. The proliferated cells correspond broadly with the cell types enumerated in a preceding paper (*2*); they include luminal cells, basal cells, myoepithelial cells, and pale cells. The epitheliosis derives from a single-layered epithelium by centripetal or centrifugal growth, the centrifugal type being more usual. The predominant cells probably correspond, as a rule, with basal cells (figs. 5 and 6) but on occasion are of different types. An outer rim of dark-staining cells encircles some tubules (fig. 6). Shay, Harris, and Gruenstein (*10*) illustrate similar formations in mammary tumors of rats and identify the dark peripheral cells as myoepithelial, an interpretation with which I agree. The myoepithelial cells grow inward in varied numbers to intermingle with the basal cells and sometimes replace them, so that elongated or oat-shaped cells fill the tubule (fig. 8). Luminal cells, distinguishable from the basal, sometimes form a regular layer lining the lumen or a palisaded, enveloping layer of the tubule. Pale cells of uncertain derivation intermingle with the basal cells. Occasionally large, pale cells with large, vesicular nuclei are especially conspicuous and fill the tubule or make up the whole of its wall (fig. 7). Differentiation of a secreting epithelium is uncommon in plaques; it will be referred to in a subsequent paper. Squamous differentiation with keratinization is rare in plaques from high-incidence strains of mice,

only 6 examples being found among some 200 plaques; but it is frequent, almost constant, in small growths of the shape and size of plaques that were obtained from mice of the low-incidence strains. The squamous plaques will receive attention in a future paper devoted to squamous differentiation in mammary tumors.

Progression

Two main forms of progression are recognizable histologically, namely, a *focal* type, which is the more common, and a *lobular* or *diffuse* type.

Focal Progression

Focal progression occurs in either central or peripheral zones of plaques. Histologically the altered focus is plainly different from the surrounding plaque and it is usually a *varied* carcinoma, which is often cystic and hemorrhagic, even when of minute size, and visible to the naked eye as a dark spot. The carcinoma stands out clearly in regressed plaques and usually distinctly, if less conspicuously, in actively growing plaques (figs. 35 and 36). Progression is sometimes unifocal (figs. 35–38) but often multifocal (figs. 58–61). The series of about 200 specimens includes examples of all stages of development, from a minute focus of carcinoma wholly enclosed in a plaque (fig. 35) to a large carcinoma with vestiges of plaque-like tissue at the periphery (fig. 39). The vestigial plaque is usually conical in shape when the carcinoma is of moderate size and there are often conical caps at opposite poles (fig. 38). As the tumor grows, it usually retains a spherical shape and bulges and distorts the plaque outline.

The growth of tumors previously designated *responsive types II* and *III* is represented graphically by ascending curves which can be resolved into a straight line with superimposed curves (*1*). In the light of the histologic findings it is possible that the straight line denotes the steady growth of a carcinoma situated in a plaque whose waxing and waning in response to reproduction accounts for the superimposed waves, but this interpretation is not applicable to those tumors which reach progressively higher peaks in successive pregnancies yet regress completely in between. During intermissions of breeding, foci of progression are enclosed in a sclerotic and poorly vascularized plaque. The enhanced vascularization of pregnancy may allow the altered tissue to acquire an adequate blood supply, which it afterwards maintains, and so may account for the "trigger action" whereby pregnancy often discloses progression that occurred earlier (*1*).

Lobular or Diffuse Progression

Lobular or diffuse progression is recognizable with greater difficulty and often only in partially regressed plaques because it does not necessarily entail evident departure from the structure typical of growing plaques. Histologic distinctions become apparent in nonpregnant mice when, for

example, one lobe retains the structure of a growing plaque whereas all the rest of the plaque has regressed (fig. 40); progression from responsiveness to unresponsiveness has occurred in 1 lobule of a plaque without notable change in histologic structure. Similarly, in a plaque in which focal progression to carcinoma has occurred, the plaque surviving at one pole of the carcinoma is typical of pregnancy, whereas that at the opposite pole is regressed and inert (fig. 41). In one portion of the plaque there is a diffuse change in responsiveness without noteworthy change in histologic structure, whereas in another portion there is focal change to carcinoma with radically altered histologic structure. In other growths, diffuse progression leads to varied degrees of modification of the internal plaque architecture without change of the external plaque form; individual tubules sometimes retain the structure of those of plaques but often diverge widely from it, especially in the direction of cribriform intratubular carcinoma, with great enlargement of the tubules and derived cords, which nevertheless preserve their sharp outlines (figs. 50 and 51). The extent of diffuse progression is likewise highly varied. In the plaque illustrated in figure 42 more than half is changed; at one extremity there is typical carcinoma but elsewhere the plaque architecture, although considerably modified, is not entirely lost. The irregular outline of the altered tissue within an undistorted plaque outline contrasts with the examples of focal progression that yields spherical growths which bulge and deform the plaque. Diffuse progression is sometimes subtotal, only vestiges of an unaltered plaque remaining (fig. 44); the retention of the plaque outline and shape again contrasts with the distortion resulting from focal progression when only plaque vestiges survive. Diffuse progression involves the transformation of all pre-existing tubules covering a small or wide area of the plaque; focal progression involves displacement of pre-existing tubules by progressive growth from a single and primarily small focus. The extreme case of total diffuse progression is difficult to establish because no vestiges remain to prove the antecedent existence of a plaque. The inference of total progression rests mainly on the complete retention of plaque shape and outline, the retention, to varied degrees, of the internal organoid architecture of plaques, and the observation of a graded series of lesions in which the extent of diffuse progression ranges from lobular (fig. 40) through extensive (figs. 42 and 43) to subtotal (fig. 44). Total progression is the most plausible explanation of the numerous small "tumors" of plaque size and shape to which reference is made below. It accounts, possibly, for giant plaques, although the information about the circumstances of origin, development, and potentialities of most giant plaques is inadequate for confident interpretation. Total progression from responsiveness to unresponsiveness without modification of plaque architecture or tubular detail is rare, but at least 2 *unresponsive* plaques of typical structure, yet derived from nonpregnant mice, seem genuine examples.

Diffuse progression is not uncommon in the remnant of plaque capping a tumor that originated by focal progression; the plaque architec-

ture remains, but the tubules take on the characteristics of intratubular carcinoma (figs. 41, 50, and 51). Multiple independent secondary foci may develop in the surviving plaque tissues. When the secondary foci are numerous throughout the plaque tissue, the distinction between focal and diffuse progression becomes tenuous.

The Origins of Tumors

"Plaque" and "nodule" are primarily anatomical terms, and there are several histologic varieties of nodules (*4,5*) as there are of plaques; but, despite notable exceptions, plaques and nodules broadly represent early stages of neoplasia whence a later stage of "tumor" emerges by progression. In this section "tumor" applies loosely and sometimes evasively to growths that are not plainly nodules or plaques and that bear marks of a more advanced biological stage of neoplasia. The term is used here irrespective of the size of the growths and includes some that in size and outward form resemble plaques or nodules. Tumors, within this meaning, originate in hyperplastic nodules, in plaques, within ducts, or in apparently unchanged mammary tissue. The majority of tumors in my series originate in plaques. More systematic search would no doubt multiply the examples of other modes of origin without discounting the preponderance of plaque origin.

Origin in Plaques

The evidence for the origin of the majority of tumors from plaques rests on the previously published observations that the development of unresponsive tumors from responsive plaques can be followed clinically (*1*) and on the histologic demonstration, now reported, of progression within plaques.

The tumors resulting from focal progression are usually *varied* tumors, which are often cystic and hemorrhagic (figs. 37–39). The frequency of the less easily recognized diffuse progression is not known. Several kinds of tumors probably originate by diffuse progression, intratubular carcinoma being the most frequent and characteristic outcome. The plaque form and even the internal plaque architecture are not at first abolished. Many small tumors in my series have the size and shape of plaques, and they were recorded as such during life or *post mortem*. Some of the tumors retain much of the internal architecture of plaques, although the individual tubules correspond with those of intratubular or irregular tubular carcinoma (figs. 45, 46, 50, and 51). The plaque architecture persists in more modified but still distinct form in some tumors but is lacking in others. The preservation of plaque form is remarkable. Various forms of incomplete and imperfect plaques, including half-plaques, are matched by tumors of similar shape and size (figs. 47–49). The correspondence in form is hardly attributable to chance. Total progression of a pre-existing plaque is the most plausible explanation of the plaque form.

Direct Origin

Many small tumors show no evidence of origin from nodules or by focal or diffuse progression within plaques. No vestiges of plaque or nodule are visible, although, since serial sections throughout the whole growth are not available, their complete absence is not proved. The tumors are smaller than many plaques and lack the plaque form and none of them has an organoid, plaque-like structure; they are spherical or irregular in shape; histologically, they are less *varied*, hemorrhagic, and cystic than the tumors originating by focal progression within plaques (fig. 52). Some are pure, or almost pure, microtubular growths and others are of irregular tubular type. These small tumors are often remarkably compact or dense; the small spindle or oat cells of which they consist are so closely packed that no orderly arrangement can be distinguished. In view of the various peculiarities, a direct origin from mammary tubules is probable. Several earlier reports mention the direct origin of tumors without an intermediate stage of hyperplasia (*11,12*).

Origin in Nodules

Of the few hyperplastic nodules found by chance in random sections some are secreting, some sclerotic, and some of the "inflammatory" or regressing type (*7,13*). Five small tumors seem to have originated in nodules, of which traces survive at the periphery (fig. 53); the nodular hyperplasia is secreting in 1 case and sclerotic in 1.

Origin in Ducts

Clear evidence of intraductal origin is scarce. Carcinoma sometimes fills one segment of a duct or, when more extensive, appears in sections as a chain or plaque-like aggregation of intraductal carcinomas (figs. 54–57). Judging from the few available examples, intraductal carcinoma is apt to be of the "comedo" type (figs. 56 and 57). In the earlier stages, epithelial overgrowth may project into the duct or outward from it into the surrounding breast tissue. Both kinds of growth may occur simultaneously (fig. 55), and there is no histologic difference between them. There is probably no consistent difference in histologic type between tumors originating inside ducts and those originating outside. The "comedo" modification is reasonably attributable to growth within a confined space with a restricted blood supply.

Multicentric Origin

Examples of multicentric origin in plaques and in ducts have already been mentioned (figs. 54–61). With continued growth, the multiple foci coalesce to form 1 tumor of composite structure. The steps in this fusion are shown in various examples of multifocal progression in plaques, and large tumors often show evidence of their composite structure. Multicentric origin is one factor contributing to histologic complexity in mammary tumors. The tumors resulting from multifocal progression are

often, but not necessarily, of similar histologic type (fig. 59). Greater histologic complexity is likely to result when focal and diffuse progression occur side by side, yielding tumors of different types (figs. 60 and 61), and when focal progression to *varied* carcinoma is followed by diffuse progression to intratubular carcinoma in the remainder of the plaque (figs. 41 and 42).

Discussion

I have not found a clear description of a typical plaque in previous accounts of mammary neoplasia in mice. Apolant (*11*) and Haaland (*14*) illustrate lesions which resemble plaques, and some references to "adenoma" or "fibroadenoma," rare in mice, possibly allude to plaques. Gardner (*4*) records growth of tumors during pregnancy and regression after parturition in 5 mice, of which 3 eventually died with adenocarcinoma; but the tumors were not examined histologically during the responsive phase. It is unlikely that plaques with the distinctive structure and behavior now described would escape notice if they developed often in most strains of mice; more probably they are common only in certain strains. Some of the incomplete and imperfect plaques, however, resemble, and perhaps are identical with, previously recorded lesions, especially the "diffuse" and "ductular" types of hyperplasia. Goormaghtigh and Amerlinck (*6*) and Pullinger (*9*) describe diffuse nodules composed of tubules that grow to considerable length and terminate, sometimes, in expansions like the end-bulbs of normal development. Pullinger comments on transitions between "diffuse nodules" and "ductular hyperplasia," which Gardner (*4,5*) describes as a dense overgrowth of small branching ducts and alveolar processes. One of Gardner's illustrations, showing a small carcinoma within an area of ductular hyperplasia, suggests focal progression in a regressing plaque. Huseby and Bittner (*7*) describe nodules composed of fine ducts, often branched, radiating from a relatively small focus to give the appearance of a many-pointed star. One lesion in my material seems to conform with this description (fig. 24). Later, Huseby and Bittner (*8*) record extensive development of very fine ducts occupying a large proportion of the mammary fat pad but devoid of radial orientation. Fekete (*15*) describes nodules with radially arranged tubules having terminal end-bulbs, and 2 or 3 of her illustrations show tubules like those in growing plaques. The characteristic feature of almost all the examples of early neoplasia in my material is a system of branching tubules, often with bulbous ends, which forms a unit of structure. Rarely, the units are solitary, and rather less rarely they are loosely and irregularly associated in *imperfect* plaques, which probably correspond with some of the above-mentioned lesions recorded by others, but predominantly they are associated in an orderly way to make up an ellipsoidal growth of macroscopic size. The peculiarity of the material is not in the units of structure but in the organization of the units to form a symmetrical structure of relatively large size. The mechanism of this organization is not clear. Huseby and Bittner reasonably attribute their star-shaped lesions

to growth radiating from a small initial focus. The plaque form probably implies multiple outgrowths from relatively long stretches of ducts. The multilobular plaques beneath the nipple seemingly involve several branches of a primary duct (figs. 16–18). The symmetry of the discs is less easily explained. Although the divisions of the mammary fat pad possibly influence the shape of the lesions, the "half-plaques" and other incomplete forms show that the shape of the lesion is not fully determined by the space available for its unimpeded growth. Extrinsic mechanical factors evidently are subsidiary to an intrinsically determined habit of growth.

Ordinarily, plaques are about 10 times as big as nodules, but there is no evidence that nodules grow into plaques. All the observations indicate that plaques start as plaques. According to my interpretation, nodules and plaques are alternative, not consecutive, stages of development. The special interest of plaques is that by reason of their large size they allow clinical observation of a biological stage of neoplasia more usually reached in nodules that are inaccessible to observation in the living animal. In particular, plaques give exceptionally favorable opportunities for the study of responsiveness and progression during early neoplasia. There are some indications—which are scarce, owing to the inaccessibility of nodules to clinical observation—that nodules behave like plaques. Some nodules, like plaques, are probably dependent upon hormonal stimulation for continued growth and, although some undergo progression to hormone independence, others regress temporarily or permanently (*4,13*).

The present observations disclose the histologic basis of responsiveness and progression but leave many complexities unresolved. The prime difficulty in interpretation springs from the lack of reliable histologic signs of responsiveness or of imminent progression. During pregnancy it is impossible to predict what will happen after parturition; the only test of responsiveness is what *does* happen. During a short space of time after parturition, unequivocal evidence of regression is visible before the histologic structure existing a few hours earlier, in the final stage of pregnancy, is wholly obscured. Observations at this time confirm the inferences about the sequence of events drawn from the examination of large numbers of plaques at various stages of development. They also show the limits of safe inference during pregnancy, for they demonstrate an unanticipated responsiveness in some complex growths hardly distinguishable from invasive carcinoma. The superficially attractive method of serial biopsy has not been used in this investigation on account of uncertainties about the effects of biopsy on subsequent development and about the homogeneity of the lesions, upon which the reliability of the method depends. The former uncertainties remain but no doubt remains, in the light of the histologic findings, about the frequent lack of homogeneity, especially after progression has begun. With this material serial biopsies could give dangerously misleading results; they do not provide a reliable escape from the laborious examination of numerous specimens taken at well-chosen stages of the sequence.

The basic feature of growth during pregnancy is a radial outgrowth of hyperplastic branching tubules from a core of simple tubules which change little or not at all. The hyperplasia seems to involve predominantly, but not exclusively, epithelial cells of "basal" type. In responsive tumors, epithelial degeneration begins at about the time of parturition and advances quickly, accompanied by a reduction in vascularity and changes in the connective-tissue stroma. Lesions which remain visible at *post-mortem* examination during intermissions of breeding contain irregular shrunken tubules in a fibrous stroma. The precise fate of lesions which regress so far that they cannot be found *post mortem* is not known; presumably there is a return to normal or nearly normal breast structure, although there is a permanent biological alteration, because, as previous clinical observations have shown (*1*), the lesions almost invariably recur promptly at the same site when the mouse next becomes pregnant.

Two chief modes of progression are recognizable, namely, *focal* and *diffuse* types. The outcome of focal progression, as a rule, is a tumor of commonplace type, conspicuously different in histologic structure from the surrounding plaque. Diffuse progression, especially when total and involving the whole plaque, is more difficult to follow. There are indications, needing further study, that total diffuse progression can occur without histologic change, the result being an unresponsive plaque differing from the parent plaque only in the capacity to grow without the stimulus of pregnancy. Some "giant plaques" probably originate in this way.

Most tumors in this series originate in plaques, as shown by the clinical history and histologic observations on focal and diffuse progression. Three other modes of origin are recognizable, namely, by development in hyperplastic nodules or within ducts and by direct development from unaltered mammary tissue. The infrequency of origin in hyperplastic nodules compared with its preponderating frequency in some other strains of mice (*4,7,13*) is explicable by the argument advanced earlier that plaques and nodules represent alternative paths of development. Genetic constitution probably determines the choice between the 2 paths.

The fragmentary observations on the relationship between mode of origin and histologic type of tumor allow only tentative conclusions requiring much further investigation. The most usual outcome of focal progression in plaques is irregular tubular or varied carcinoma, often complicated by cyst formation and hemorrhage. Diffuse progression leads more commonly to intracanalicular or intratubular carcinoma, which often retains, in some degree, the characteristic architecture of plaques. Conspicuously organoid tumors are mostly intratubular carcinomas of special kinds and will be discussed separately in another paper. Direct origin seems especially prone to give microtubular carcinoma or irregular and undifferentiated carcinomas of an unusually compact type. The observations on intraductal origin are too scanty to justify detailed discussion. It is pertinent to note, however, that origin within ducts is often claimed on the basis of inferences from relatively far-advanced

lesions and not on direct observation of the initial stages. True intraductal origin is probably rare. Multicentric origin, which is common in plaques, is an evident source of complexity in definitive tumors. The separate foci of growth merge ultimately to make up a composite tumor, different lobes of which may be widely disparate in structure. Multifocal progression in plaques often yields multiple tumors of similar histologic type but differences in type are sometimes apparent. Greater complexity is likely to result from the side-by-side occurrence of focal and diffuse progression, which yield tumors of distinctly different histologic types. The varied types of epithelial differentiation seen in plaques recur, often more conspicuously, in tumors; subsequent papers are planned to describe their manifestations at various stages of mammary neoplasia.

Discussion of the origin of tumors is hampered by the inadequacy of current terminology, which cannot cope with the diverse combinations of unit characters that, within limits, are independently variable and liable to independent progression. According to my view "plaques," "nodules," and "tumors" all represent stages in a process of mammary neoplasia that advances, in different directions and degrees, by progression in 1 or more characters. Responsiveness is one of the characters liable to independent progression. Ordinarily in my material responsiveness combines with the distinctive type of histologic structure seen in plaques, but the correlation between responsiveness and structure is far from complete. Some tumors with plaque-like architecture are unresponsive, and some responsive tumors have the histologic criteria of malignant tumors and scarcely a trace of plaque architecture.

Summary

In the mice upon which these observations are based the most usual manifestation of early neoplasia is a disc or plaque of macroscopic size, made up histologically of symmetrically arranged systems of branching tubules. During pregnancy the tubules grow out radially from a relatively unchanged central area and branch repeatedly, with hyperplasia of their epithelium. After parturition the hyperplastic epithelium degenerates and the plaque either disappears altogether or contracts to a much smaller disc having a peripheral zone of shrunken tubules in a sclerotic stroma.

Two main forms of progression are recognizable histologically, namely, *ocal progression*—leading most frequently to *varied* carcinomas, which are often cystic and hemorrhagic, and *lobular* or *diffuse progression*, which is especially prone to yield *intratubular* carcinoma. Progression within plaques is the main sources of definitive tumors in the present material, but some tumors originate in hyperplastic nodules, within ducts, or directly from apparently normal mammary gland.

Evidence is given to show that hyperplastic nodules and plaques are alternative, not consecutive, steps in mammary neoplasia and correspond with similar biological stages of development. The peculiarity of the present material is not in the units of which early lesions are composed

but in the organization of the units to form symmetrical lesions, which, on account of their relatively large size, are amenable to clinical investigation and so are particularly favorable for the study of responsiveness and progression during early stages of mammary neoplasia.

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PLATES

PLATE 53

FIGURE 1.—Plaque deep to the panniculus carnosus, in the third mamma of a late-pregnant mouse. Tubules radiate from a central "medulla" to make a more compact "cortex." (Strain BA F_1.) × 15

FIGURE 2.—A similar plaque with more adipose tissue in the medulla, situated superficial to the panniculus carnosus in the second mamma of a late-pregnant mouse. (Strain BR6 F_3.) × 15

FIGURE 3.—The edge of a plaque from a late-pregnant mouse, showing branching tubules and hyperplastic end-bulbs. (Strain BR4 pink F_3.) × 60

FIGURE 4.—Tubules and end-bulbs at the edge of a plaque from another late-pregnant mouse. (Strain BR4 blue F_4.) × 320

FIGURE 5.—Tubules in a plaque from a late-pregnant mouse. Dark and pale cells and dividing cells are present in the hyperplastic tissue. The eccentric lumen, due to uneven asymmetrical hyperplasia of the tubule wall, is characteristic. (Strain RB F_1.) × 320

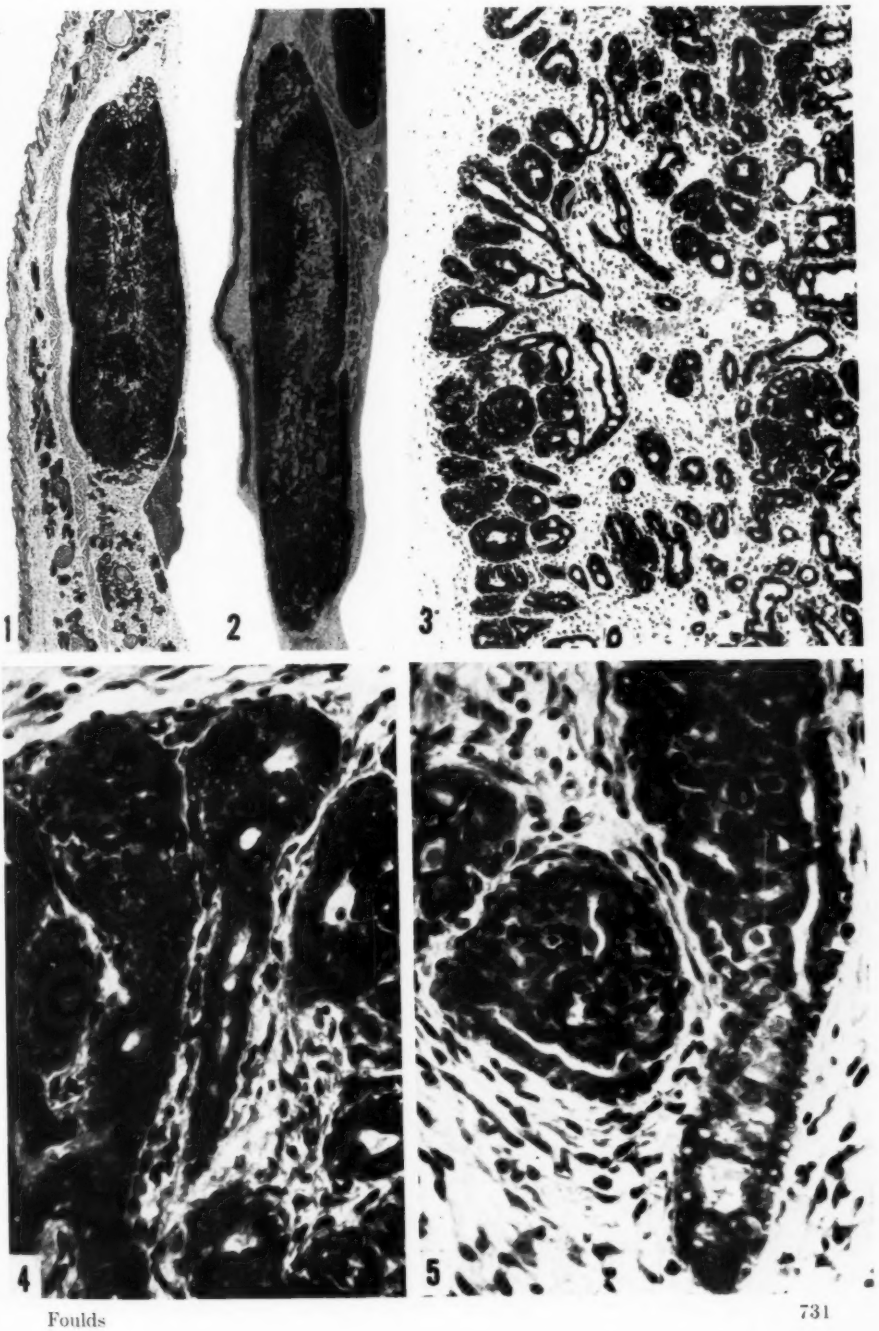

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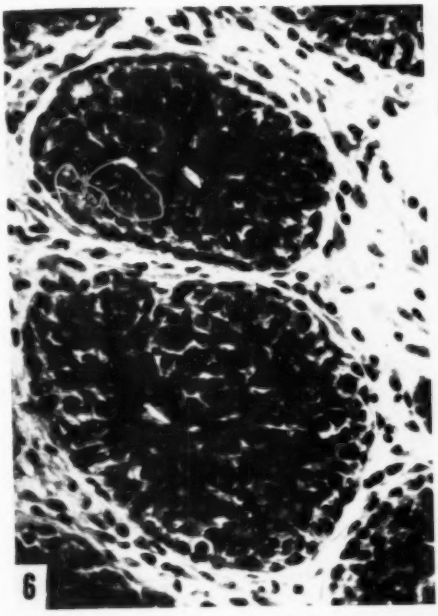
PLATE 54

FIGURE 6.—Two hyperplastic tubules, cut transversely, in a plaque from a late-pregnant mouse. The distinct enveloping layer of smaller cells is, possibly, myo-epithelial. (Strain BR F_1.) × 320

FIGURE 7.—The edge of a plaque from a pregnant mouse, with closely packed tubules in which pale cells are unusually abundant. (Strain BR4 blue F_3.) × 320

FIGURE 8.—Elongated cells in a hyperplastic tubule from a plaque in a late-pregnant mouse. (Strain RB F_1.) × 320

FIGURE 9.—Regressing plaque from a mouse killed soon after parturition. There is karyorrhexis and cytoplasmic vacuolation in the tubular epithelium. The stroma is loose and edematous. (Strain BR6 F_{20}.) × 320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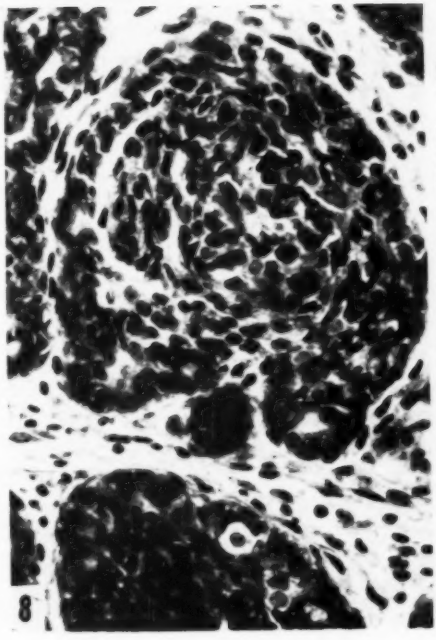
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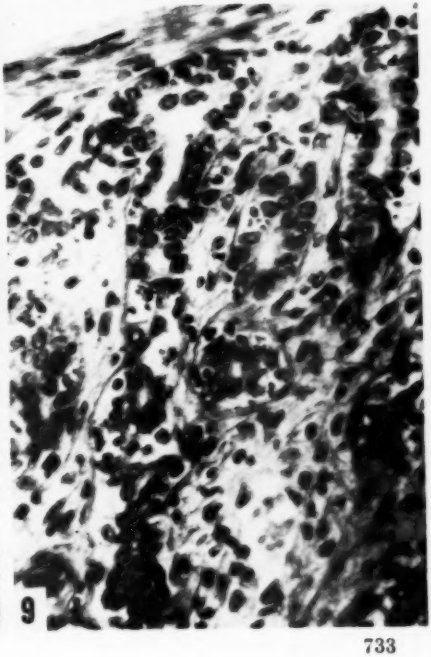
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PLATE 55

FIGURE 10.—Regressing plaque from a mouse killed soon after parturition. There is less nuclear debris than in figure 9 but greater vacuolation of cytoplasm, with the characteristic "ballooning" of the peripheral cells of tubules, as indicated by *arrows;* the tubules are more widely separated by stroma, in which there is early fibrosis. (Strain RB F_1.) × 320

FIGURE 11.—A less usual form of regression with shedding of degenerated cells into the lumen. Peripheral degeneration of tubular epithelium is also present but less conspicuous than in figures 9 and 10. (Strain RB F_1.) × 320

FIGURE 12.—A portion (about one half) of a regressing plaque, from a mouse which littered 5 days previously, with fibrosis of the stroma between the shrunken tubules. (Strain RB F_1.) × 60

FIGURE 13.—Regressed plaque with sclerotic stroma from a mouse which littered 6 days previously. The irregularity of the *left* border of the plaque indicates invasion of the panniculus carnosus. (Strain BR4 blue F_2.) × 15

FIGURE 14.—A regressed plaque, from a nonpregnant mouse, showing a thick cortex with dense sclerosis and a central fatty medulla. (Strain RB F_1.) × 15

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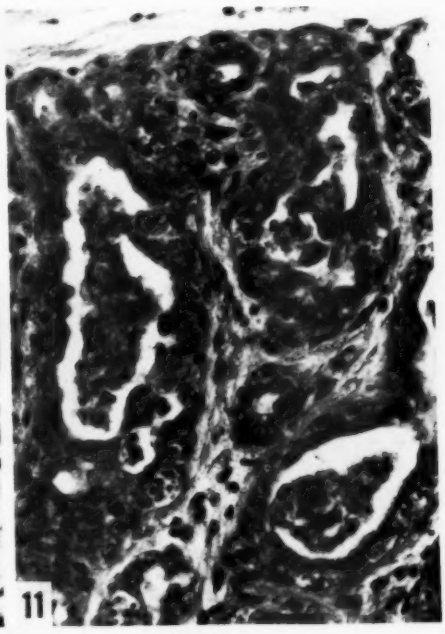
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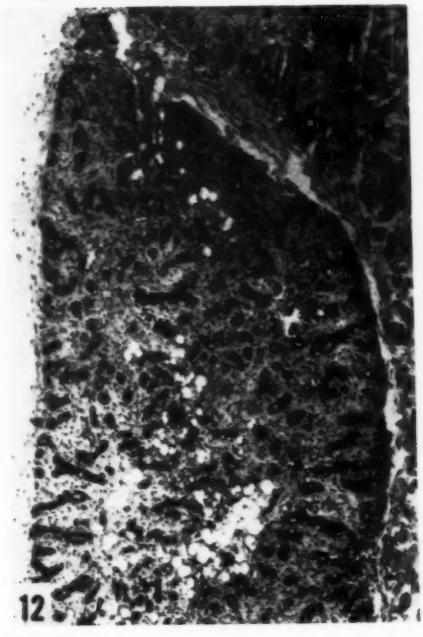
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PLATE 56

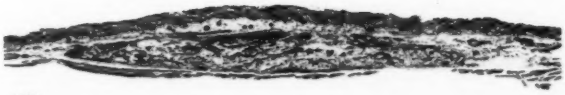
FIGURE 15.—A regressed plaque from a mouse which had not been pregnant for over 8 months. The lesion, found at autopsy and showing scanty and patchy fibrosis, is at the site of a tumor that reached a maximum size of 1.0 × 0.5 cm. during the last pregnancy and then regressed and was not palpable again. (Strain BR4 pink F_7.) × 15

FIGURES 16 TO 18.—Three compound plaques similarly placed beneath and around a nipple. × 15

Figure 16 shows a plaque near the peak of growth from a mouse which littered 3 hours before the tumor was excised. (Strain BR6 F_2.)

Figure 17 illustrates a regressing tumor from a mouse that had littered 5 days previously. (Strain RB F_1.)

Figure 18 shows a later stage of regression with more advanced fibrosis in a tumor from a mouse killed 9 days after parturition. The darkly stained area is a focus of progression to carcinoma. (Strain BR4 blue F_2.)



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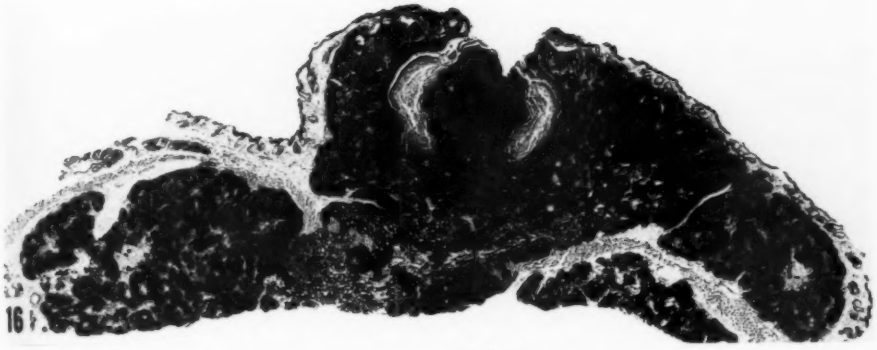

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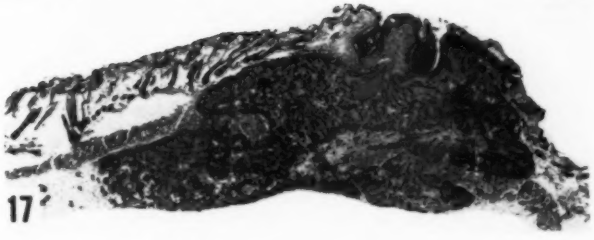

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PLATE 57

FIGURE 19.—Incomplete plaque. Adipose tissue with patchy tubular hyperplasia intervenes between compact areas of hyperplasia corresponding with the limits of the usual form of complete plaque. (Strain BR4 blue F_2.) × 15

FIGURE 20.—Incomplete plaque with focal progression to carcinoma at the *upper* edge. In the *lower* portion there is patchy development of plaque units (shown under higher magnification in fig. 27) and in the *center* an area of adipose tissue almost free from tubular hyperplasia. (Strain BR4 blue F_2.) × 15

FIGURE 21.—Incomplete plaque forming about one half of a complete plaque. The incomplete end is sharply defined. (Strain RB F_1.) × 15

FIGURE 22.—Incomplete plaque. About two thirds of the usual plaque form is developed and, by contrast with figure 21, the incomplete end is not sharp. (Strain RB F_1.) × 15

FIGURE 23.—Incomplete plaque. The wedge-shaped area of tubular hyperplasia, with its base toward the skin surface and with a mammary duct at its apex, corresponds to a segment of a complete plaque. (Strain BR4 blue F_3.) × 15

FIGURE 24.—Imperfect plaque or diffuse nodule made up of units like those in plaques but radiating from a single focus. (Strain BR4 pink F_3.) × 15

FIGURE 25.—A somewhat similar imperfect plaque or diffuse nodule with a focus of cystic carcinoma at the *right* border. (Strain BR4 pink F_3.) × 15

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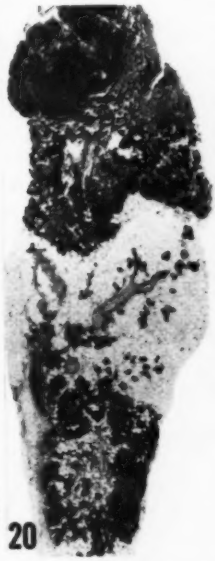
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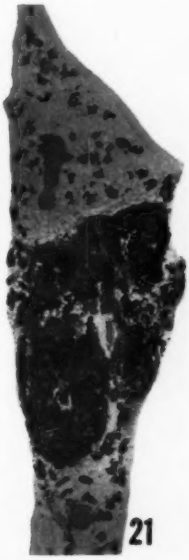
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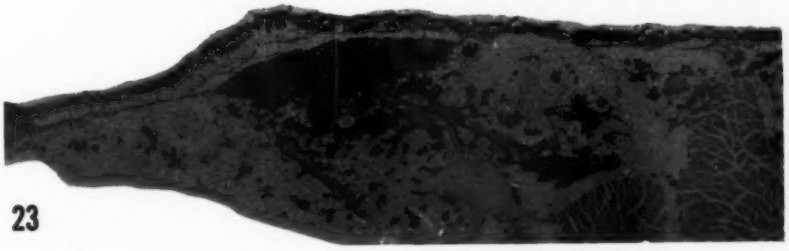
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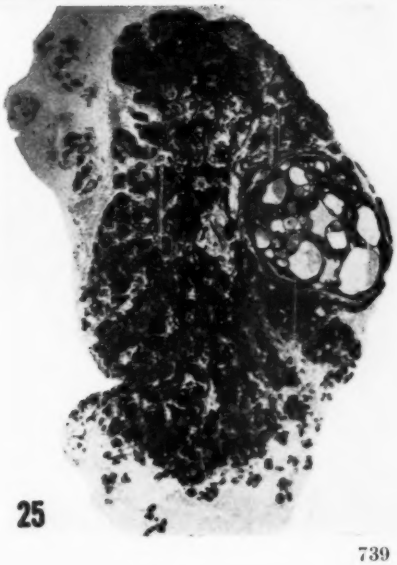
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PLATE 58

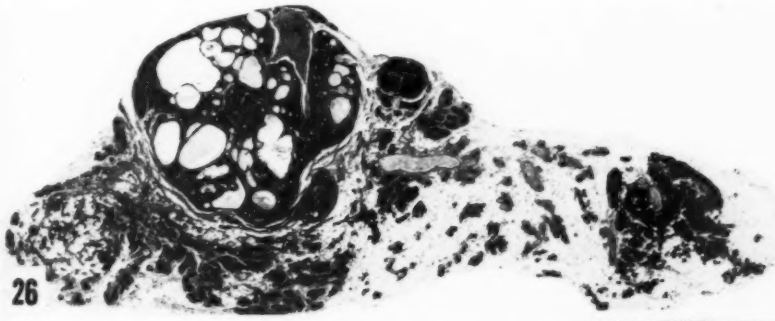
FIGURE 26.—Imperfect plaque with an area of carcinoma. In the *right* portion there is an outlying area of plaque units where the branching tubular structure is recognizable at this low magnification. (Strain RB F_1.) × 15

FIGURE 27.—Unit of branching tubules from the incomplete plaque illustrated at lower magnification in figure 20. (Strain BR4 F_2.) × 60

FIGURE 28.—Plaque of typical structure but of unusually small size. (Strain BR4 blue F_3.) × 15

FIGURE 29.—Giant plaque with clear distinction between cortex and medulla. (Strain BR6 F_3.) × 15

FIGURE 30.—Giant plaque. A complex multilobular growth. (Strain BR F_1.) × 15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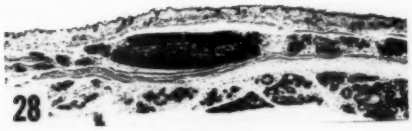
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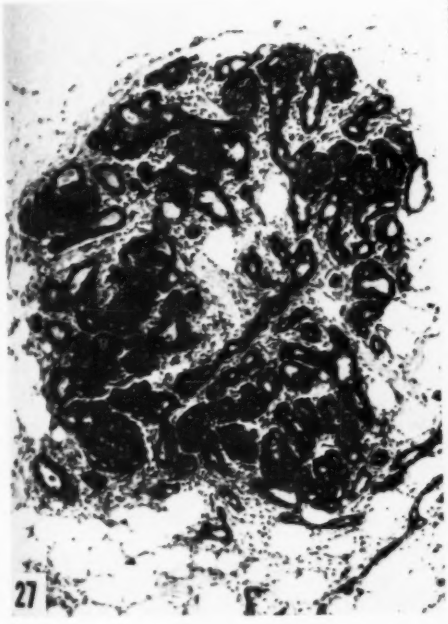
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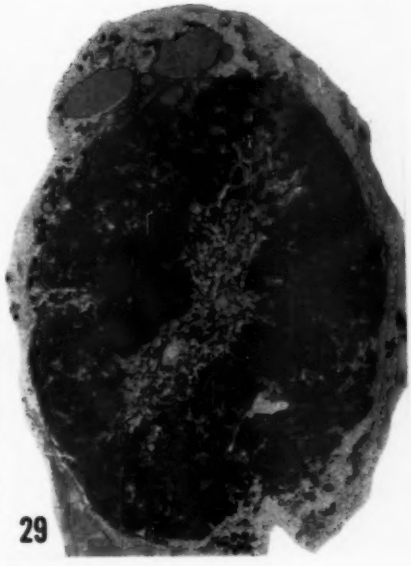
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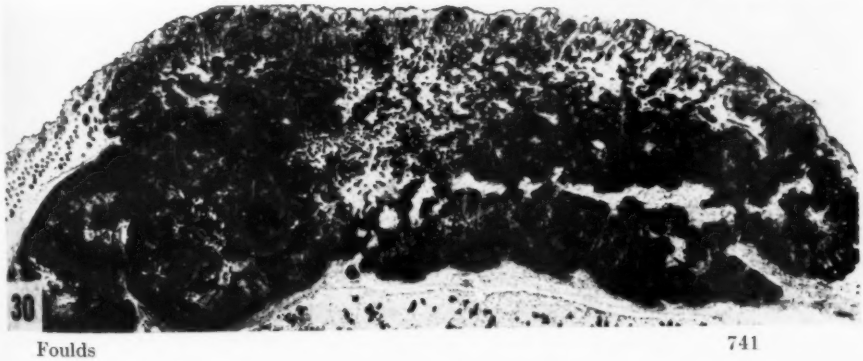
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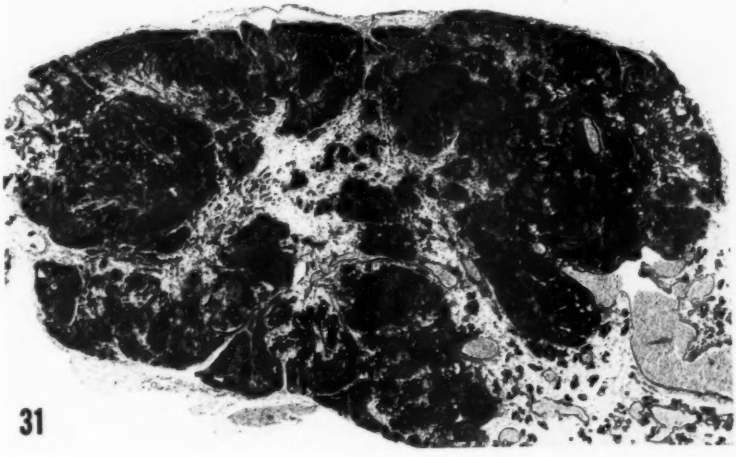
PLATE 59

FIGURE 31.—Irregular complex plaque. The units resemble those of ordinary plaques but they are aggregated less regularly to make a larger growth in which the plaque architecture is obscured. (Strain BR4 blue F_2.) × 15

FIGURE 32.—Thin, superficial regressed plaque from a nonpregnant mouse with progression to carcinoma at the *right*. The plaque tubules are predominantly longitudinal. The epidermal changes extend far beyond the carcinoma to the limits of the plaque. (Strain BR6 F_3.) × 15

FIGURE 33.—A portion of the lesion illustrated in figure 32 showing epidermal thickening, subepidermal tubules, fusions of epidermis and tubules, and 1 sinus (*arrow*). × 60

FIGURE 34.—Superficial regressed plaque involving the dermis. The plaque tubules mostly run parallel with the skin surface but some of the more superficial ones bend toward it. The overlying epidermis is thickened. (Strain RB F_1.) × 60



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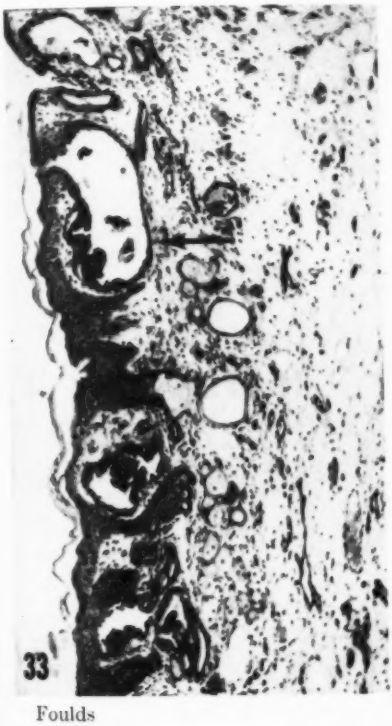

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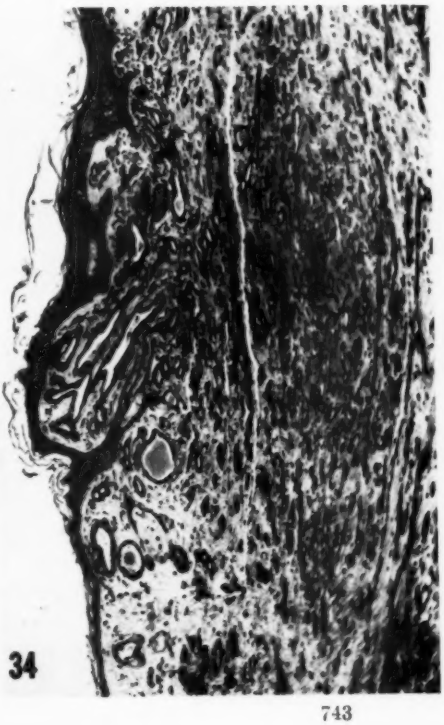

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PLATE 60

FIGURES 35 TO 39.—Focal progression in plaques.

(Other examples of focal progression are illustrated in figures 18, 25, 26, 41, 58, 59, and 60.)

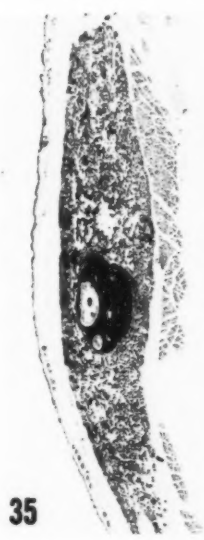
Figure 35.—Centrally placed focal progression to carcinoma within a regressing plaque. (Strain BR6 F_3.) × 15

Figure 36.—Larger focus of carcinoma in an active plaque. (Strain BR6 F_3.) × 15

Figure 37.—Cystic carcinoma with conical cap of regressed plaque. (Strain BR4 blue F_2.) × 15

Figure 38.—Carcinoma with circular outline in a regressed giant plaque. (Strain BR4 blue F_3.) × 15

Figure 39.—Carcinoma with residual plaque forming a small cap at the *right* border. (Strain BR4 blue F_4.) × 15



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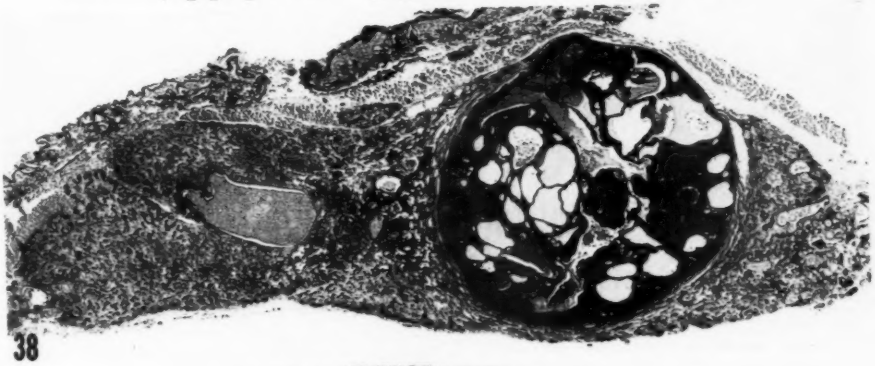

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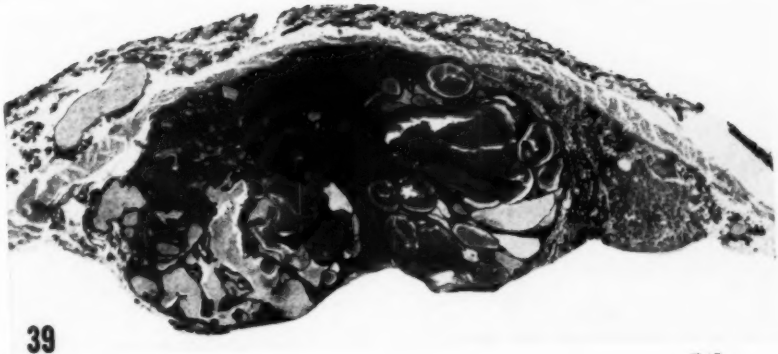

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PLATE 61

FIGURE 40.—Lobular progression evidenced by the persistence of one lobe of an otherwise regressed plaque. (Strain BR6 F_2.) × 15

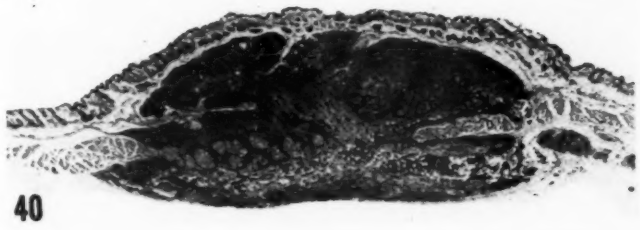
FIGURE 41.—Central focal progression to carcinoma with secondary diffuse progression in the *left* portion of the regressed plaque while the *right* portion remains inactive. (Strain BR6 F_3.) × 15

FIGURE 42.—Focal progression to cystic carcinoma at the *left* margin with diffuse progression to tubular carcinoma retaining some plaque characteristics in a portion of the remainder of the regressed plaque. (Strain BA F_1.) × 15

FIGURE 43.—Plaque with extensive progression to carcinoma, especially at the *lateral* margins. (Strain RB F_1.) × 15

FIGURE 44.—Subtotal diffuse progression in a plaque, remnants of which are visible at the *right* margin. (Strain BR4 blue F_2.) ×15

FIGURE 45.—Plaque-shaped carcinoma, probably attributable to total diffuse progression in a plaque of which no remnant is recognizable in this section. (Strain RB F_1.) × 15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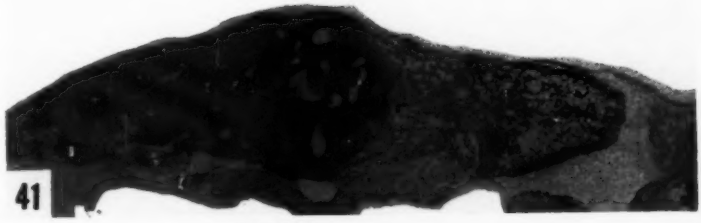
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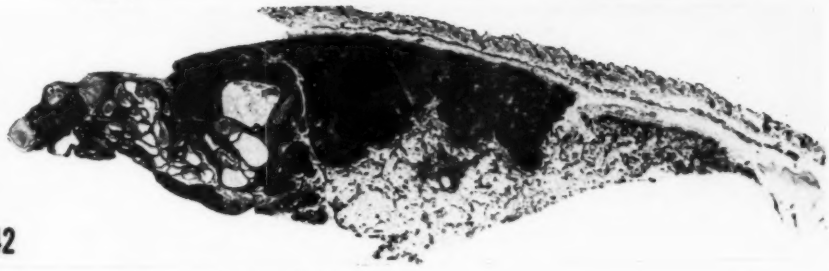
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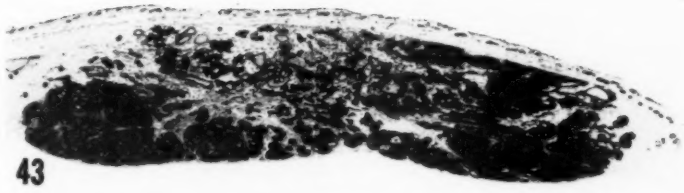
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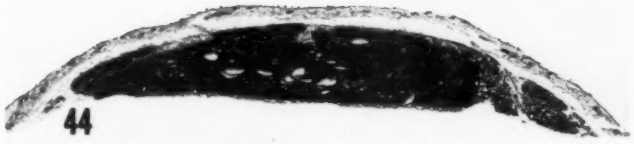
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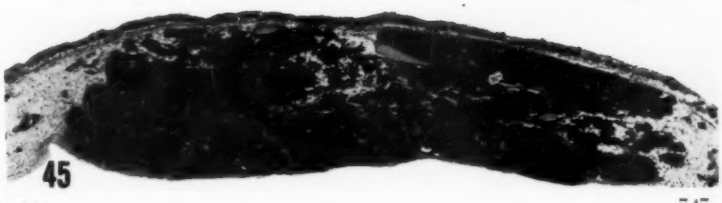
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PLATE 62

FIGURE 46.—Plaque-shaped carcinoma with traces of the internal architecture of a plaque. (Strain MIII.) × 15

FIGURES 47 AND 48.—Lesion resembling an imperfect plaque, but the tubules resemble those of intratubular carcinoma. (Strain BR6 F_{27}.) × 15 and × 60

FIGURE 49.—Carcinoma with the outward form of an incomplete plaque. (Strain BR6 F_{31}.) × 15

FIGURES 50 AND 51.—Plaque-shaped carcinoma. The tubules at the *upper* margin of figure 50 have a plaque-like arrangement but individually, as shown in figure 51, they have the characteristics of intracanalicular carcinoma. (Strain BR4 pink F_3.) × 15 and × 60

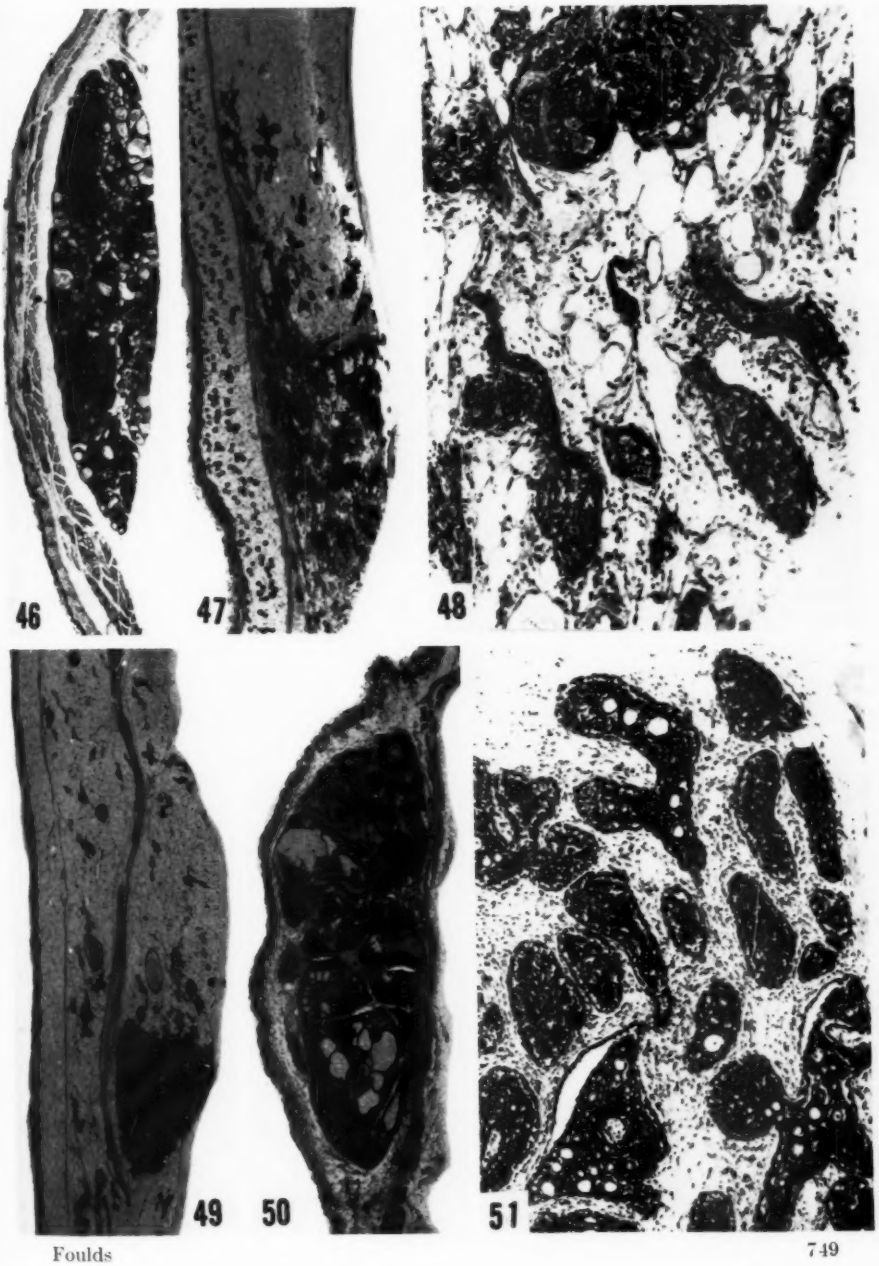
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PLATE 63

FIGURE 52.—Small carcinoma without clear evidence of contiguous plaque or nodule. More commonly the small "direct" tumors are compact or dense growths resembling the type illustrated in figure 53. (Strain BR6 F_3.) × 15

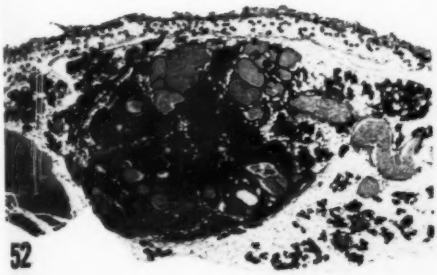
FIGURE 53.—Small microtubular carcinoma with traces of nodular hyperplasia at its periphery. (Strain BR4 blue F_{16}.) × 15

FIGURE 54.—Multiple intraductal carcinomas. (Strain BR4 pink F_9.) × 15

FIGURE 55.—An area of figure 54 under higher magnification showing, *below*, intraductal growth and, *above*, the continuity of growth of similar type into, and outward from, the duct. × 60

FIGURE 56.—Multiple intraductal carcinomas forming a chain. (Strain BR4 pink F_4.) × 15

FIGURE 57.—Higher magnification of the lower extremity of figure 56 showing the "comedo" type of carcinoma. × 60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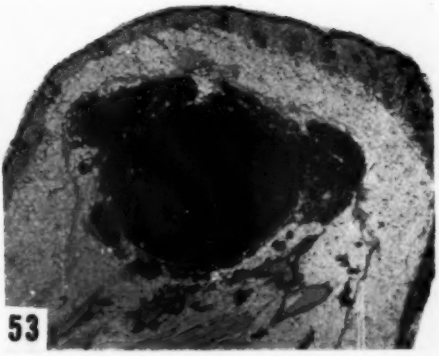
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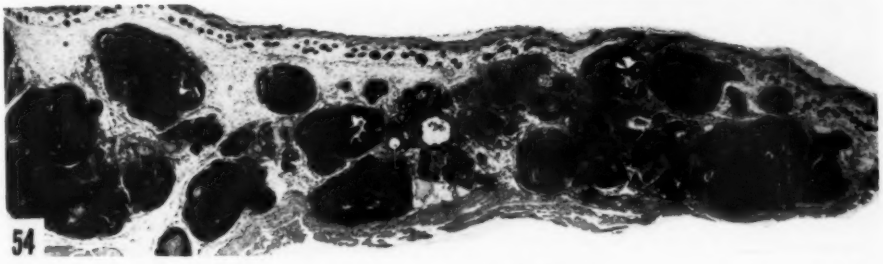
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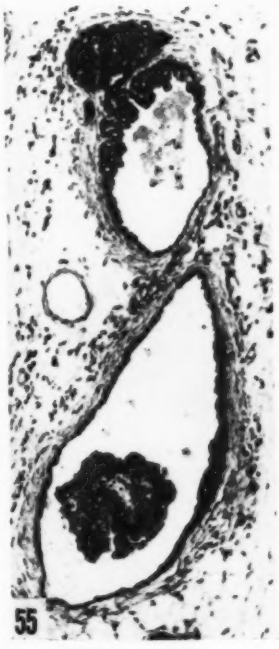
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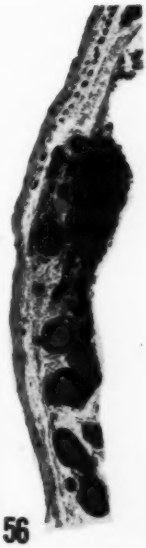
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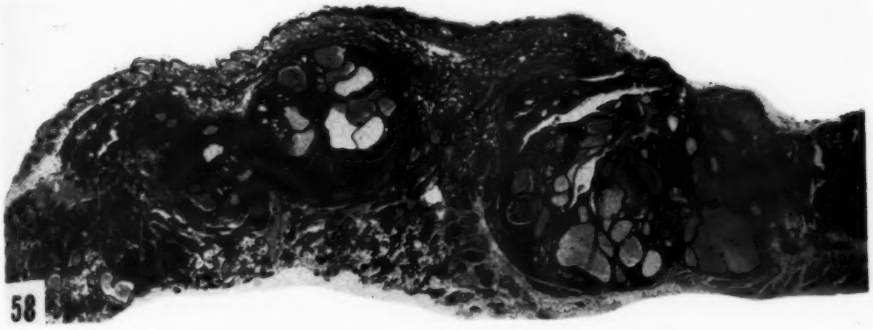
PLATE 64

FIGURE 58.—Multiple focal progression in a giant plaque. (Strain BR4 blue F_5.) × 15

FIGURE 59.—Two dissimilar tumors with regressed plaque intervening. The tumor to the *left* shows evidence of recent secretion. (Strain RB F_1.) × 15

FIGURES 60 AND 61.—Varied types of progression within a plaque. Higher magnification in figure 61 of the marked area of figure 60 shows, at the *lower left* corner, the edge of a cystic carcinoma and above it an uncommon type of intratubular carcinoma; surviving plaque tissue occupies the *right* border. (Strain BR4 blue F_4.) × 15 and × 60



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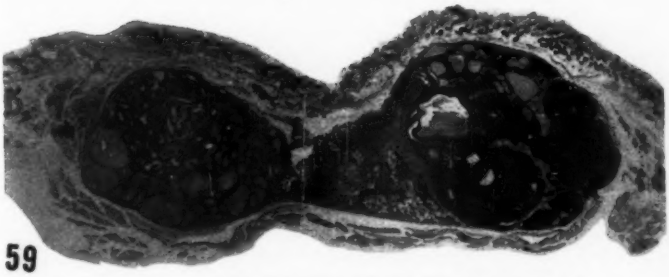

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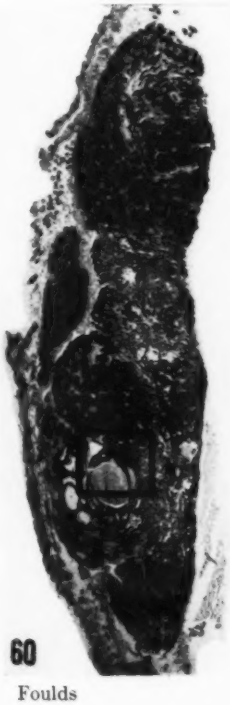

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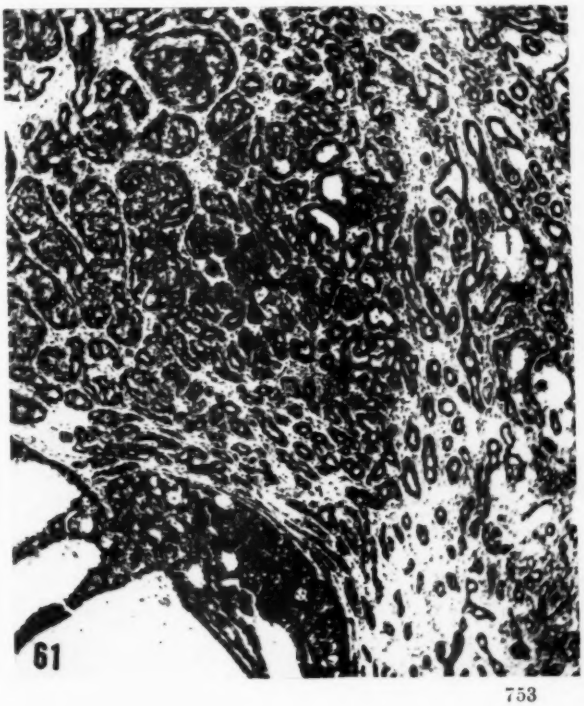

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The Histologic Analysis of Mammary Tumors of Mice. III. Organoid Tumors[1, 2]

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The fundamentally organoid structure of tumors, asserted by Albrecht half a century ago, has been discussed by Nicholson (*1*) and Böhmig (*2*), who give references to the early German literature. Nicholson maintains that the majority of tumors, if not all, are organoid—in the sense of Albrecht; they are built up on the same plan as the tissues in which they originate, their cells are grouped to form tissues and organs in a way that is sometimes remarkable, and their structure, growth, and development are intelligible only if tumors are viewed as aborted organs.

The present paper deals with organoid structure in mammary neoplasia in mice and especially with the comparatively rare tumors in which an organoid pattern is predominant.

Material and Methods

The material and methods of this investigation were those described previously (*3*). Special methods such as tracings and wax-plate reconstructions from serial sections, as used by Böhmig and his colleagues (*2*), were not employed. The organoid structures here described are visible in random sections.

Types of Organoid Growth

Organoid structure in definitive tumors is, in essentials, the manifestation in late stages of neoplasia of habits of growth which, ordinarily, are conspicuous only in transitory early stages and, most notably, in the plaques already described (*4*). Plaques are symmetrical associations of units which radiate from a central core or medulla; the units are branching tubules which, beginning with a small bore and a single-layered epithelial wall, run a straight course, with increasing diameter and epithelial hyperplasia, to end in pyriform expansions or end-bulbs. The most conspicu-

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ously organoid tumors are made up, similarly, of straight branching units with terminal expansions. In the simplest organoid growths, conveniently termed *simple radiate* tumors, tubules or solid cords radiate symmetrically from a single growth center. *Molluscoid* tumors (*5,6*), the only hitherto named organoid mammary tumors of mice, are simple radiate tumors complicated by progressive keratinization. Simple radiate tumors are rare. More complex tumors, here named *compound organoid* tumors, are built of similar units but grow from multiple centers, and a radiate structure is less conspicuous or absent. Less distinctive organoid growth is recognizable in *intratubular* carcinoma, in *fissured* and *macroglandular* tumors, in *irregular tubular* and *microtubular* carcinomas, and in transplanted tumors.

Simple Radiate Tumors

It is difficult or impossible rigidly to separate radiate tumors from plaques and giant plaques (*4*) but 3 growths, of which 2 are illustrated (figs. 1–4), are judged to fall outside the reasonable limits of definition of plaque or giant plaque. The common feature is the radial outgrowth of branching tubules and solid cords from a single center. In the tumor not illustrated the radially disposed units begin centrally as fine tubules and expand into solid cords with many of the features of intratubular carcinoma. The tumor illustrated in figure 1 has a conspicuous central or medullary area in which are tubules of narrow bore walled by 2-layered epithelium and solid epithelial cords of similar size. Well-defined branching cords of loosely packed epithelial cells radiate from the center toward the periphery. They branch and increase in thickness toward the periphery where they end in expansions, most of which are hollow—due probably to degeneration of central cells (fig. 3). Mitotic figures are present, especially in the end-bulbs, which at one edge of the section clearly invade muscle. The tumor is plainly radiate and organoid except in one region where there is an area of dilated tubules containing inspissated material—probably the result of antecedent secretion—and a contiguous area of compact, irregular tubular carcinoma without radial architecture. The third tumor is likewise plainly radiate, especially at the periphery (fig. 2). The radiating, branching units have characteristics not seen plainly in any other organoid tumor, namely, those of intracanalicular growth (fig. 4). The units are outlined by a layer of pale cuboidal or columnar cells within which are darker cells, sometimes completely filling the tubule and sometimes leaving a central lumen bordered by a multilayered epithelium. Mitotic figures are abundant in both pale and dark cells. Within the dark-celled cores of the units there are often distinct whorling of cells and some traces of squamous differentiation, but no keratinization. At one edge there is invasion of muscle. As in the previously described tumor, there is a region devoid of radial architecture where multilayered epithelium lines clefts—giving the appearance of macroglandular carcinoma and also less differentiated carcinoma without distinctive features.

Compound Organoid Tumors

In the more complex tumors developing from multiple growth centers the constituent units are similar to those in the simple radiate tumors but their radiate disposition is less consistent or sometimes indiscernible. Of the 6 tumors in this group the most important, a transplantable tumor, Yx 30, which I owe to my former colleague Dr. B. D. Pullinger, will be described first and in greatest detail. I am indebted to Dr. Pullinger for a copy of her notes, for histologic material, and for transplanted tumors from which serial transplantation was continued.

The primary tumor developed on the left side of the thorax of a year-old breeding female of the agent-free RIII strain. It tripled in size within 2 weeks; it was then excised but recurred within 3 weeks, and the mouse was killed with an infiltrating tumor a month later. Dr. Pullinger successfully transplanted material from the primary and recurrent tumors and one line was continued in RIII male mice up to the 5th passage when it was abandoned owing to change of laboratory. The transplanted tumors grew rapidly in almost all the inoculated mice.

Histologic material is available from the primary, recurrent, and 19 transplanted tumors. All the tumors have essentially the same histologic structure and all contain some areas of radiate organoid growth (figs. 5–10). Tubules of narrow bore radiate from multiple growth centers. In the primary and recurrent growths they are usually walled by 2 layers of epithelium, but some are filled with epithelium to make solid cords. In the transplated tumors the finest tubules are more consistently walled by 2-layered epithelium and often grow extensively without expansion or hyperplasia to make up large portions of the growth (figs. 8–10). These tubules grow to a considerable length and branch often; they are sometimes straight, but often curved and ramified, and as a rule a radiate disposition is apparent only peripherally, where the fine tubules expand into solid cords as a result of epithelial hyperplasia (figs. 6 and 7). The cells in the cords are closely packed; there is some whorling in the cores but no true epithelial pearls or keratinization. The epithelial cords branch, even after great thickening, and terminate in end-bulbs which are usually solid; the termination is sharp in outline and usually rounded but sometimes bifurcate (fig. 6). Mitotic figures are abundant, especially in the end-bulbs, which are invasive (figs. 6 and 7). The invading tips retain their sharp outlines; they shed no free invasive cells. The invading epithelial cords do not, in general, follow lines of least resistance but maintain their radial course; in some places the cords cut across muscle fibers at right angles (fig. 10).

The organoid and basically radiate structure of Yx 30 is evident in all sections of primary and transplanted tumors, but in many areas, large and small, it is obscured by irregular epithelial proliferation. The irregular proliferation is especially conspicuous when it occurs in areas of extensive growth of fine tubules (fig. 9); it is less evident but similar in kind when it proceeds in areas with wider, solid, and more closely packed epithelial

cords (figs. 5 and 8). It often produces areas of undifferentiated carcinoma without distinctive features. In some areas, however, patent tubules are conspicuous within the carcinoma, which is similar to the *fissured* type of Apolant. Judging from the results of transplantation the modification of structure is not the result of progression; it is a variant involving no permanent qualitative change. The significant change is probably from wholly centripetal, intratubular proliferation in the organoid growth to centrifugal extratubular proliferation, which leaves the lumina patent but abolishes the clear boundaries between contiguous units.

The other 5 tumors of the group are of the same basic type as Yx 30 but differ from it in detail. The radiate structure is less pronounced; sometimes it is apparent only where the units impinge on large blood vessels (fig. 11) and sometimes it is hardly discernible, almost all the units being seen in cross section (fig. 12). In every section of Yx 30 the radiate structure is recognizable somewhere; it is unlikely that misfortune in the orientation of sections wholly accounts for the difficulty of finding it in the other tumors. Moreover, these tumors lack the extensive development of fine tubules often seen in Yx 30; the course of fine tubules is often short and they quickly thicken into broader cords which, mostly, are filled with loosely packed epithelial cells (figs. 13 and 14). The cords are often cribriform and the central cells are frequently arranged in whorls or circlets, a common occurrence in ordinary intratubular carcinomas. Sometimes there is central degeneration in the cords and traces of the earliest phases of keratinization, but there are no true epithelial pearls or outright squamous differentiation. The end-bulbs are varied; some are solid, as in Yx 30, but in many the cells are less tightly packed and may enclose a lumen. In 1 tumor most of the end-bulbs have a lumen, which is lined by one or more layers of epithelial cells and often contains desquamated epithelial cells. Mitotic figures are abundant, especially in the end-bulbs. Invasion of muscle and subcutaneous tissues is conspicuous. All the tumors contain organoid units of branching tubules broadening to end-bulbs but, as a rule, the simple tubules from which the units develop do not grow as much in length as they do in Yx 30 before changing into thicker solid cords.

All the tumors of this group were large, measuring as a rule 2.0 to 2.5 cm. in their longest diameters. All for which adequate records are available grew fast—at about the same speed as the primary Yx 30, which tripled in size within 2 weeks; one tumor, as an example, increased in superficial dimensions from 0.8 × 0.7 cm. to 2.5 × 2.0 cm. in 13 days. These are probably the fastest-growing of all mammary tumors of mice. They are also highly invasive—2 of the present series grew within the thigh muscles. Metastasis was not seen.

All the tumors except Yx 30 grew in mice of high-incidence strains. At death the tumor-bearing mice ranged from 11 to 14 months in age. One mouse was a virgin; the others were breeders. Two mice were litter mates aged 13 and 14 months, respectively, at death.

Keratinizing Radiate Tumors (Molluscoid Type)

Haaland (*5,6*) described 5 tumors, all in the mammary region, with "... a peculiar structure which places them in a group by themselves." He adopted Borrel's term "tumeur molluscoide," based on the radiate structure recalling that of *Molluscum contagiosum*. Long, straight cylinders radiate from the center of the tumor. Centrally the cylinders are wholly keratinized; peripherally they are cellular for a short distance and end in bulbs often forming "cysts" or "acini" lined by one or more layers of epithelium, with desquamated ephithelial cells free in the lumen. Jobling (*7*) and Slye, Holmes, and Wells (*8*) report single molluscoid tumors and Andervont and Dunn (*9,10*) record 4, but Teutschlaender (*11*) has published the only detailed description, based on 2 tumors.

My series includes 6 tumors which I consider molluscoid and 7 others in which molluscoid-type growth is present but not predominant. It is not clear how strictly the criteria for the recognition of molluscoid tumor have been applied previously; judging from the illustrations, Jobling's tumor and 2 of Andervont and Dunn's (*9*) are dubious examples and belong more probably to my second group, which is intermediate between typical molluscoid tumors and adenoacanthomas. Another tumor of Andervont and Dunn's (*10*), with minimal central keratinization, resembles tumor Yx 30 rather than a true molluscoid tumor. According to Haaland's original descriptions and illustrations there are 2 essential criteria, namely, radiate structure and progressive keratinization.

In 2 of my tumors the molluscoid structure is almost perfect (figs. 15 and 16) and in the other 4 it is unmistakable, if rather less regular (fig. 17). The rays of the tumor are conspicuously branched (fig. 16). Although Haaland makes no specific mention of branching it is evident in his drawing (*6*). Teutschlaender emphasizes dichotomous branching, which he demonstrated in one tumor by wax-plate reconstructions. Keratinization is complete in the central portions and no living epithelial cells remain. It extends outward as the tumor grows, tapering into a central core within a cylinder of epithelial cells. The cylinder often contracts slightly where keratinization stops, broadens again, and ends usually in a terminal expansion or bulb, which is usually rounded, or as Teutschlaender describes, sometimes heart-shaped or bifurcate (fig. 18). Some end-bulbs are composed of loose masses of epithelial cells, of which the outermost layer is palisaded. There are fine clefts among the inner cells but intercellular bridges are not visible. Spaces of varied sizes are present in other end-bulbs; they are lined by one or more layers of epithelial cells and often contain desquamated cells, as described by Haaland and by Teutschlaender. These end-bulbs match those present in the organoid tumors described in the preceding section although they are not usually so compact as the most common form of end-bulb in Yx 30. Mitotic figures are numerous in the end-bulbs, which are the invasive growing tips of the tumor. The present observations confirm those of

Haaland and of Teutschlaender on the invasive, destructive growth of molluscoid tumors. The radiate organoid structure is maintained at the invading edge and, as remarked by Teutschlaender, it is not attributable to growth along lines of least resistance. The invading tips retain their sharp outlines; there is no dissociation or shedding of invasive isolated cells. I have not seen metastasis, and the only recorded example, by Haaland, of metastasis to the mediastinal areolar tissue is not, without more detailed information, a convincing example. The rim of proliferating tumor tissue is rarely wide and is sometimes extremely thin (fig. 15). It is evident that progressive keratinization keeps pace with the progressive growth of the tumor and, to this extent, the tumors are self-limiting. The records, where available, show that the rate of growth is moderate, exceeding that of most adenoacanthomas but falling short of that of the nonkeratinizing organoid tumors described in the preceding section. Whereas the nonkeratinizing tumors triple their size in about 2 weeks, molluscoid tumors do so in about 2 months.

All my molluscoid tumors grew in mice of low-incidence strains and all but 1 in mice of the inbred line BR0, believed free of the milk agent. The age of the tumor-bearing mice at death ranged from 17 to 26½ months. I did not attempt transplantation of molluscoid tumors, having failed to recognize them during life or at *post-mortem* examination. None of Haaland's implantations into more than 1,000 mice was successful but he had only random-bred animals. Transplantation within inbred lines might well succeed. A diagnosis of molluscoid tumor is suggested by the relatively fast invasive growth of a keratinizing tumor in a middle-aged or old mouse of a low-incidence strain.

The tumors which are not wholly molluscoid developed similarly in mice of low-incidence strains. They differ from true molluscoid tumors mainly in the imperfection and inconstancy of the radiate architecture. Usually some portions resemble commonplace adenoacanthoma, and keratinization does not proceed as regularly as in typical molluscoid tumors.

Two additional tumors not included in the groups already mentioned are plainly organoid but not molluscoid. They closely resemble the nonkeratinizing organoid tumors described in the preceding section except for the keratinization, which is patchy. One tumor has a radiate architecture in some parts, but not generally, and is comparable with the Yx 30 tumor; keratinization is mostly central but irregular (fig. 19). Both tumors derive from mice of low-incidence strains, aged 16 and 20½ months, respectively, at death. They are the keratinizing counterparts, in low-incidence strains, of the nonkeratinizing organoid, but not predominantly radiate, tumors which occur in high-incidence strains, as already described.

All reported molluscoid tumors have been in the mammary region. There has been an unnecessary reluctance to admit that they develop from mammary tissue. In his first paper Haaland remarked on the resemblance of the tumors to hair bulbs, from which he thought they derived. In a later paper, however, he made no reference to histogenesis.

Jobling inferred an origin from hair follicles, and Slye, Holmes, and Wells thought that the tumors grew from the stratified portions of mammary ducts near the nipple. Teutschlaender was undecided whether molluscoid tumors developed from mammary gland or from hair follicles. There is no evidence, based on observation, in published descriptions or in my material that molluscoid tumors originate in hair follicles or in ducts near the nipple. The molluscoid tumor in low-incidence, agent-free strains of mice is the keratinizing counterpart of the simple radiate tumors, free from keratinization, in high-incidence, agent-bearing strains. The molluscoid tumor differs in 1 unit character, namely, keratinization, which is almost always present in mammary tumors in my low-incidence strains.

Intratubular Carcinoma

Intratubular carcinoma, of which the organoid tumors hitherto described are specialized types, is characterized by epithelial proliferation within a sharply defined outline. The epithelial mass is commonly cribriform, the cells are often arranged in whorls or circlets and traces of epithelial pearls and keratinization are frequent, but keratinization is rarely conspicuous in high-incidence strains of mice. Intracanalicular growth is a subdivision of intratubular growth distinguished by an outer, palisaded layer of pale-staining cuboidal or columnar cells, within which darker cells form a multilayered epithelium lining a cleft or fill the tubule completely with an epithelial mass liable to the same variations as in ordinary intratubular growth. These types of growth develop by centripetal epithelial proliferation in the tubules of plaques, as described previously (*4*), and in the tubules of the radiate and other organoid tumors described in preceding sections. Diffuse progression within plaques leads to intratubular carcinoma, which in early stages often retains to a considerable extent the plaque architecture. The early development of ordinary intratubular, as well as intracanalicular, carcinoma is evident also in simple radiate tumors (fig. 4). It is not certain that the intratubular carcinoma that is one of the commonest and most widespread components of the gross tumors in my series originates from plaques or from radiate tumors, but it has characteristics of organoid growth. Ordinarily the tumors are compact aggregations of broad cords of cells subject to the variations already described. The cords are sharply circumscribed but they are tortuous, and in general there is no evident radial architecture or distinction between stem tubules and thickened end-bulbs. Branching of the cords, however, is often visible, especially at the growing edge where a radiate architecture also is frequently apparent and even conspicuous. Sometimes tubules thickening to end-bulbs branch and fan out from a fine-stem tubule and give evidence of organoid growth in the strictest sense (fig. 20). In one intracanalicular growth the units run a long, straight course with occasional branching as thin tubules walled with 2-layered epithelium before they broaden out to form compact intracanalicular growth without distinctive pattern, except that occasionally the whole course from narrow tubule to expanded end-bulb is visible (fig. 21). This tumor links the commonplace

intracanalicular and intratubular growths with the fully organoid growths, and its exclusion from the group of organoid tumors described in the preceding section is somewhat arbitrary; it is exceptional chiefly in the long, straight course of the stem tubule.

In summary, there is substantial evidence that intratubular and intracanalicular tumors are manifestations of organoid growth, which is plainly evident in plaques and simple radiate tumors and variously complicated in other gross tumors.

Irregular Tubular and Microtubular Carcinoma

In microtubular carcinoma and in the irregular tubular areas common in *varied* tumors the tortuosity of the tubules obscures the organoid structure, which is further complicated by extratubular proliferation and by the crowding and intermingling of organoid units. Under exceptionally favorable circumstances, when the units are fairly straight and disposed in the plane of the section, a radiate organoid architecture is apparent (figs. 22 and 23).

Fissured and Macroglandular Carcinoma

Cloudman (*12*) describes a *macroglandular* type of growth characterized by clefts lined by multilayered epithelium and thinks that it probably corresponds with Apolant's *fissured* tumors ("spältenbildende Adenocarcinome" or "spältenformiges Carcinom"). Judging from the published illustrations and my own observations I believe that the 2 types are different, the distinctive feature of *fissured* tumors being centrifugal, extratubular epithelial proliferation. The tumor illustrated in Dunn's (*13*) figure 47 as a "miscellaneous tumor of an unclassified type" is possibly a *fissured* carcinoma. The important common property shared by macroglandular and fissured tumors is the sharply defined lumen, which partially compensates for the absence of a clearly circumscribed outline as a clue to organoid growth. My material provides examples of the origin of these types of growth by progression in plaques (fig. 24). Similar progression is present in a plaque from a mouse which, in another breast, had a giant plaque in which the cortical zone resembled in detail the progressed regions in the first plaque (fig. 25). Modification to these types of structure occurs also in simple radiate plaques and in the more complex transplantable organoid tumor Yx 30. One tumor showing organoid structure of a special kind is made up of units without ordered relationship to each other but sufficiently dispersed to allow their independent recognition (fig. 26). The structure of the separate units is shown mainly by the well-defined lumina. In some regions, especially at the periphery, the structure of the units is clearly evident; tubules branch and fan out from a narrow stem and end in big expansions, often exceeding in size the end-bulbs of intratubular carcinomas and due in the main to centrifugal epithelial proliferation (fig. 27). Occasionally a radiate structure is discernible at the periphery but the predominant

feature is the loose association of organoid units without an ordered pattern. Similar loosely associated units make up a plaque-shaped lesion in another mouse. The recognition of organoid units in these tumors depends on the loose association which is probably transitory; continued growth leads to fusion into a tumor in which the individual units are no longer separable, and this stage is reached, as a rule, in the macroglandular and fissured areas that are not rare in gross *varied* tumors. In the later stages of neoplasia the evidence for organoid growth rests mainly on the branchings of the lumina.

Organoid Growth in Transplanted Tumors

A preceding paragraph describes the persistence of organoid structure through repeated serial transplantations of the compound organoid tumor Yx 30. No other organoid tumor was studied so completely by transplantation. One tumor shows an organoid structure in the first and second transplant generations; the primary growth is extensively organoid, although tissue preserved from a region near that used for transplantation is not. Organoid units in the form of branching tubules radiating from a thin stem and broadening to an end-bulb are present after several passages of a tumor which at first was not evidently organoid. Close search for organoid units in transplanted tumors has not been made but their occurrence, even if rare, is significant in showing that a capacity for organoid growth is inherent in some tumor strains and persists in some strains where it is manifested under some circumstances, although not regularly present.

Discussion

The only detailed consideration of organoid growth in the mammary region of mice with which I am acquainted is that of Teutschlaender, based on 2 molluscoid tumors. Teutschlaender stresses the dichotomous branching and its resemblance to the branching of developing normal glands. My observations confirm his and disclose, similarly, the terminal bifurcations corresponding with Heidenhain's "dimeres" as described and illustrated in glandular tumors of man by Böhmig (*2*). The organoid structure of molluscoid tumors has stimulated unnecessary speculation about their histogenesis. There is no longer any force in Haaland's remark that the structure of molluscoid tumors ". . . differs so considerably from that of all other mammary tumours that there is some probability that their origin is to be looked for somewhere else than in the mammary gland." (*6*). Molluscoid tumors form but 1 conspicuous subdivision of a group of organoid tumors which have in common habits of growth that are apparent in normal development of the mammary gland and still more clearly in early mammary neoplasia, especially when it is manifested as a plaque. The units that make up organoid tumors are comparable with those that make up plaques; the units of plaques, in turn, resemble those of normal mammary development. The characteristic organoid

unit is a dichotomously branching tubule or cord stemming from a fine tubule and thickening to an expanded end-bulb, which is the growing tip. The radiate architecture of some organoid tumors matches that of plaques but it is not an indispensable criterion of organoid growth. The organoid mammary tumors retain to an extraordinary extent in late neoplasia, with gross tumor formation, the habits of growth, the differentiated units, and often the relative disposition of units that are characteristic of normal and early neoplastic mammary development.

It is noteworthy that the highly organized tumors are among the most rapidly growing and most invasive of all mammary tumors of mice. The invasiveness is not attributable to lack of adhesion and shedding of invasive free cells; the growing tips are sharply outlined. The sharp circumscription of the growing tips, although no bar to energetic invasion, is possibly correlated with the absence of metastasis.

The transplantable tumor Yx 30 decisively confirms Teutschlaender's inference from histologic examination of 2 primary molluscoid tumors that the organoid growth is an intrinsic character of the tumor and not a mode of growth imposed on the tumor by the tissues that it invades. As noted by Andervont and Dunn (*10*), the maintenance of organoid structure despite obstacles to its extension is remarkable, especially in radiate tumors. The tumors have a permanent intrinsic capacity for forming unit systems of branching tubules, arranged often in an orderly architectural pattern. Histologically and biologically, the smallest significant unit of an organoid tumor is not a "malignant cell" but a branching, differentiating neoplastic tubule.

Organoid structure is conspicuous histologically only when several exacting conditions are fulfilled. The organoid units must be recognizable individually and in large part so that their branching and differentiation of tubules from fine stem to expanded growing tip may be traced. The units must be separable by reason of their dispersion or of their orderly arrangement, as obtains, most effectively, in radiate tumors. Either the units must be sharply outlined as in intratubular carcinoma or, less satisfactorily, as in fissured and macroglandular carcinoma, a continuous distinct lumen must indicate their course. Furthermore, a substantial portion of the unit must lie in 1 plane coinciding with the plane of the section; the tubules or solid cords must run a straight or nearly straight course. These conditions are most nearly satisfied in simple radiate tumors, and, notably, in molluscoid tumors, where the sharp outlines and the prominent central keratinization combine to demonstrate the organoid architecture. Intratubular growths constitute the largest group of organoid tumors in my series, possibly because they are the least difficult to recognize. Fissured and macroglandular growth is recognizably organoid mainly, or only, when it occurs in plaques or when the units are well dispersed, which obtains chiefly in young tumors.

Primarily organoid structure becomes complicated and obscured in several ways. Progression and modulation in the undoubtedly organoid simple radiate tumors yield varied structures, including irregular tubular,

intracanalicular, fissured, macroglandular, and secreting types. In some more complex organoid tumors and in transplanted Yx 30 tumors centrifugal epithelial proliferation and the crowding and intermingling of units confuse the architecture, and some regions consist of undifferentiated carcinoma lacking a trace of organoid structure.

In view of the difficulties involved in the recognition of organoid growth and the complications and obfuscations to which it is liable, it is not surprising that conspicuously organoid structure is uncommon. The rarity of "pure" organoid tumors is probably real, depending on an unusual concurrence of independent characters; in particular, the differentiation and arrangement of units familiar in early responsive neoplasia combine with the unresponsiveness, the rapidity of growth, and the high invasiveness of malignant tumors. The individual characters are not extraordinary but their combination at a late stage of neoplasia in a single tumor is exceptional. The pure and nearly pure organoid tumors show that certain independent characters that rarely concur are nonetheless compatible. They show, too, with especial clarity the criteria by which organoid growth should be assessed.

It is difficult to decide to what extent most mammary tumors are organoid. There is evidence of organoid structure in many intratubular carcinomas and in some irregular tubular, fissured, and macroglandular growths. There is evidence also that many of the structural variants familiar in the commonplace mammary tumors originate, on occasion, by progression or modulation in organoid plaques and radiate tumors; there is no proof that they do so always, and the inference of origin from definitive structure is always hazardous. Organoid growth is certainly important in early mammary neoplasia and sometimes in late neoplasia; it is possibly, but less certainly, the basis of most or all mammary neoplasia.

Summary

Organoid structure in definitive tumors is the manifestation of habits of growth which, ordinarily, are conspicuous only in earlier transitory stages of neoplasia. Organoid tumors are made up of differentiated units which begin as tubules of narrow bore, branch dichotomously, and run a straight course with increasing diameter and epithelial proliferation to end in expanded end-bulbs, which are the growing tips. In simple radiate tumors the units radiate symmetrically from a single growth center. Molluscoid tumors are simple radiate tumors complicated by progressive keratinization, and they develop late in mice of low-incidence strains. Compound organoid tumors are composed of the same kind of units as the simple radiate tumors but they develop from multiple growth centers and the radiate architecture is less conspicuous or absent. Organoid structure is most often and most easily seen in *intratubular* growths, but less distinctive organoid growth is distinguishable also in tumors of *irregular tubular*, *microtubular*, *macroglandular*, and *fissured* types. Organoid tumors grow rapidly and are highly invasive. Pure organoid tumors, which depend upon an unusual concurrence of unit characters,

are rare. Organoid growth is conspicuous only under narrowly defined conditions and it is liable to be complicated and obscured in several ways. It is detectable, nevertheless, in some gross tumors of commonplace type and is possibly the basis of most or all mammary neoplasia in mice.

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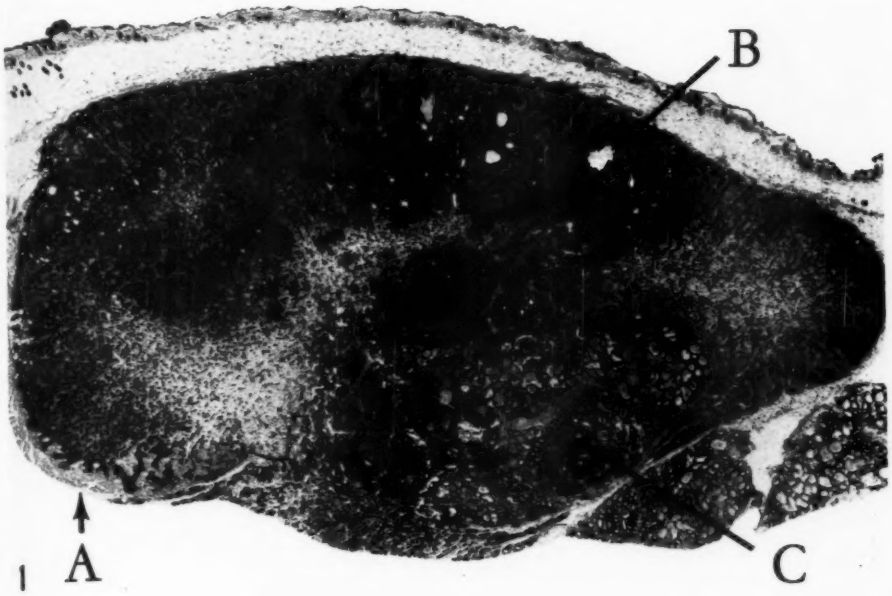
PLATE 65

FIGURE 1.—Simple radiate tumor. Invasion of muscle is present in the region marked *A*. A darkly stained area (*B*) of compact carcinoma is present in the medulla and below it is a secreting carcinoma (*C*). Figure 3 shows the *right* edge under higher magnification. (Strain BR4 blue F_{15}.) × 15

FIGURE 2.—Simple radiate tumor containing areas of fissured carcinoma indicated by *arrows*. A portion of the *upper* edge is shown under higher magnification in figure 4. (Strain RIII.) × 15



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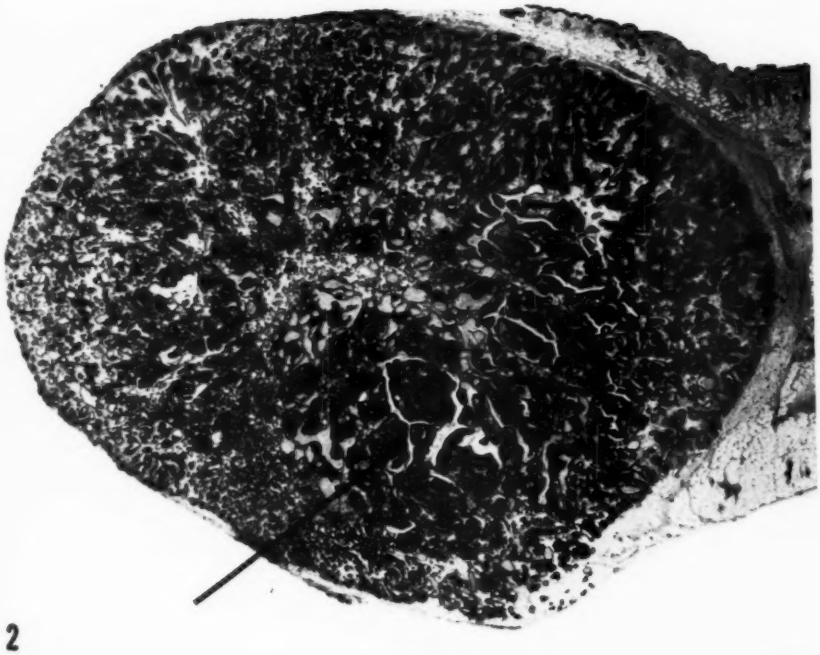

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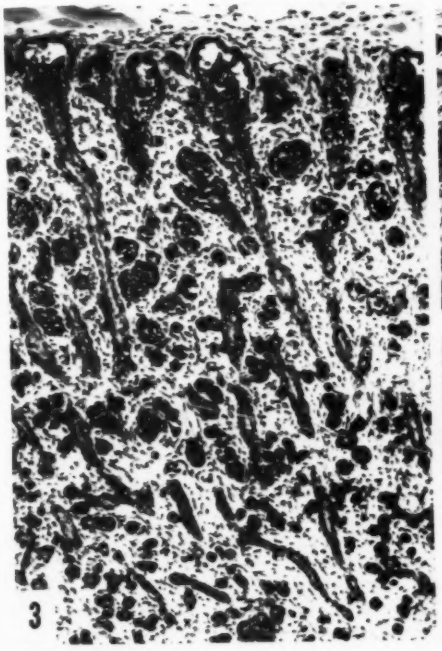
PLATE 66

FIGURE 3.—Edge of tumor illustrated in figure 1 showing the radiate branching tubules with hollow end-bulbs. × 60

FIGURE 4.—Edge of tumor illustrated in figure 2 showing the intracanalicular growth and invasion of muscle at the *upper* border. The tubules are outlined by a regular layer of pale-staining epithelium within which are irregular masses of darker-staining cells. × 60

FIGURE 5.—Compound organoid tumor, Yx 30. Biopsy specimen from the primary growth. There is a peripheral zone of hyperplastic branching tubules, a central area of narrow tubules, and, especially toward the *right* and *lower* edges, denser areas of irregular growth. × 15

FIGURE 6.—Edge of tumor shown in figure 5 with dichotomous branching of tubules which terminate in end-bulbs, 1 of which is bifurcate. × 60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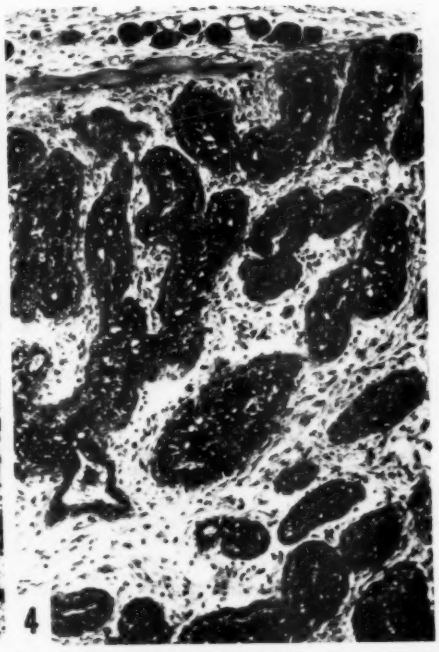
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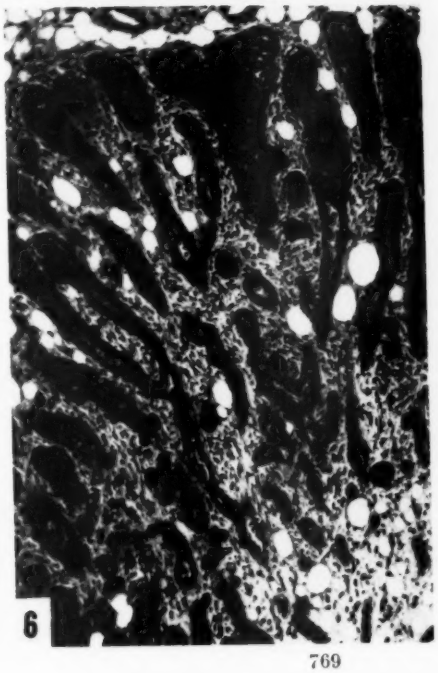
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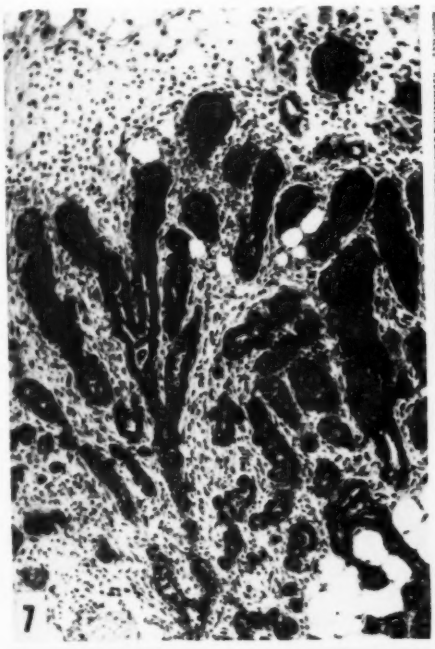
PLATE 67

FIGURE 7.—Tumor Yx 30. The edge of the recurrent growth showing the dichotomous branching of tubules with invasive end-bulbs. × 60

FIGURE 8.—Tumor Yx 30. A tumor of the third transplant generation resembles a giant plaque. Radiate growth is most conspicuous at the *upper left* border. To the *right* the growth is more irregular. Invasion of muscle is present at the *lower left* border There is an extensive medullary area of long, fine tubules. × 15

FIGURE 9.—Higher magnification of the medullary region of figure 8 shows the fine branching tubules in a loose delicate stroma and compact nodules of less regular growth. × 60

FIGURE 10.—Another region of the same tumor shows narrow tubules transecting at right angles a band of muscle which traverses the center of the field vertically. × 60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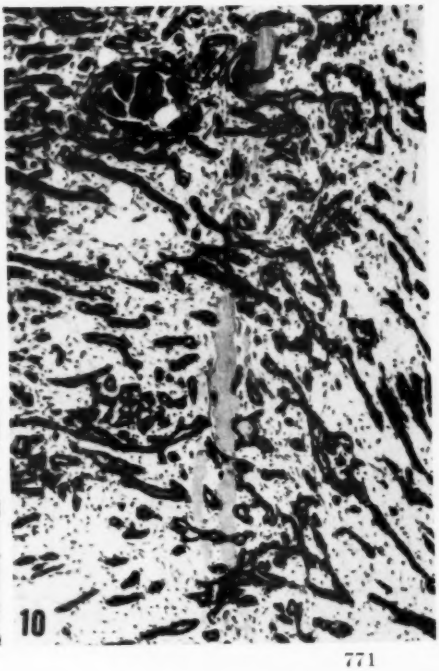
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PLATE 68

FIGURE 11.—Compound organoid tumor. The whole tumor measured 2.0 × 1.9 × 1.5 cm. Branching tubules and end-bulbs are visible adjacent to the cleft indicated by an *arrow*. Many tubules are cut transversely. Organoid structure is lost in the *lower right* portion. (Strain BR6 F_3.) × 15

FIGURE 12.—Compound organoid tumor. The whole tumor measured 2.5 × 1.5 × 1.5 cm. (Strain BR4 blue F_8.) × 15

FIGURE 13.—An area of the tumor shown in figure 12 with organoid structure. × 60

FIGURE 14.—Another compound organoid tumor with short, narrow tubules widening into tubules with inconspicuous organoid arrangement and a general resemblance to intratubular carcinoma. (Strain BR6 F_3.) × 60

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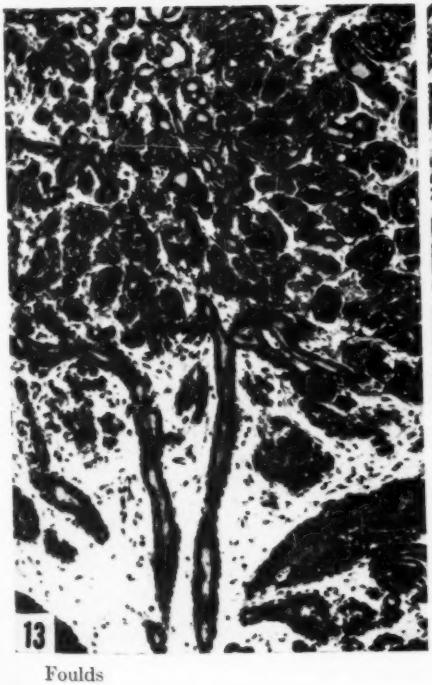
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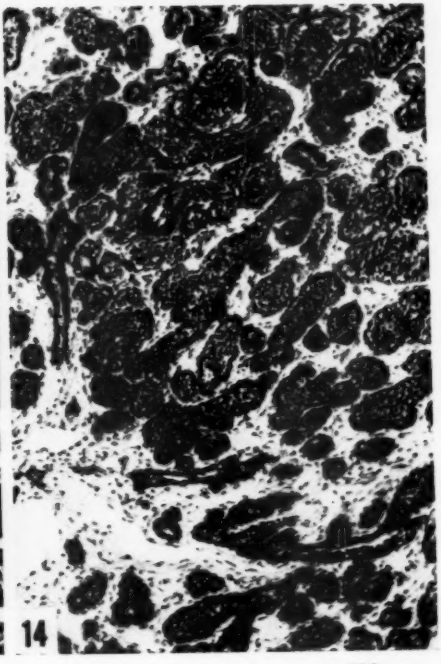
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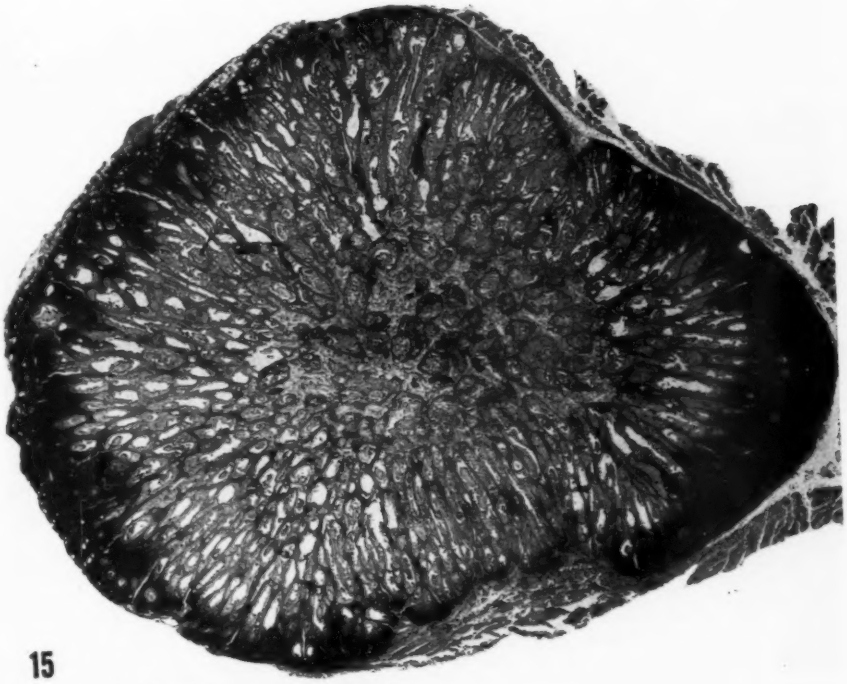
PLATE 69

FIGURE 15.—Molluscoid tumor. The growth radiates symmetrically from a central core. Progressive keratinization extends outward and reaches the periphery at some places at the *upper* and *lower* borders. The darkly stained rim of growing tumor is thickest at the *lateral* borders. (Strain BR0 F_4.) × 15

FIGURE 16.—Edge of a larger molluscoid tumor measuring 2.8 × 2.5 × 1.6 cm. (Strain BR0 F_{24}) × 15

FIGURE 17.—Imperfect molluscoid tumor. (Strain BR0 F_1.) × 15



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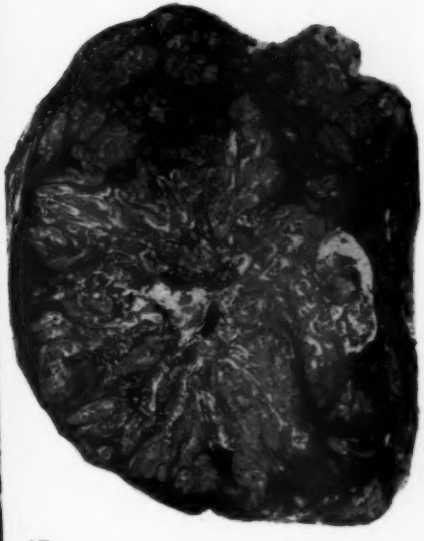

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PLATE 70

FIGURE 18.—Edge of molluscoid tumor shown in figure 15 showing keratinized tubules and end-bulbs. × 60

FIGURE 19.—Edge of a tumor with irregular distribution of keratinization. This field shows early keratinization of a tubule with multiple branches and end-bulbs. (Strain BR0 F_{26}.) × 60

FIGURE 20.—Organoid structure at the edge of an intratubular carcinoma. (Strain BR4 blue F_2.) × 60

FIGURE 21.—Organoid structure of an intracanalicular growth. (Strain MIII.) × 60

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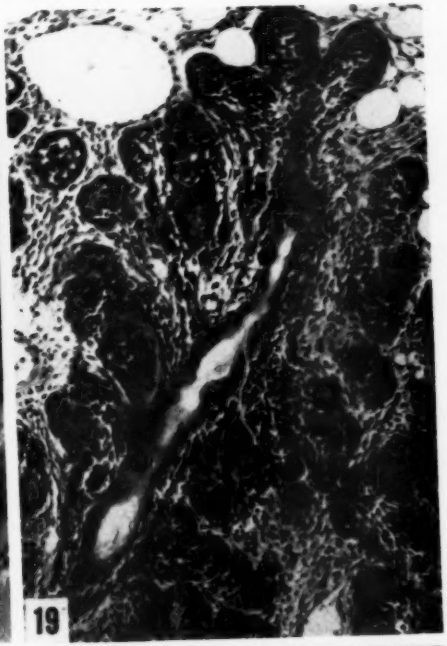
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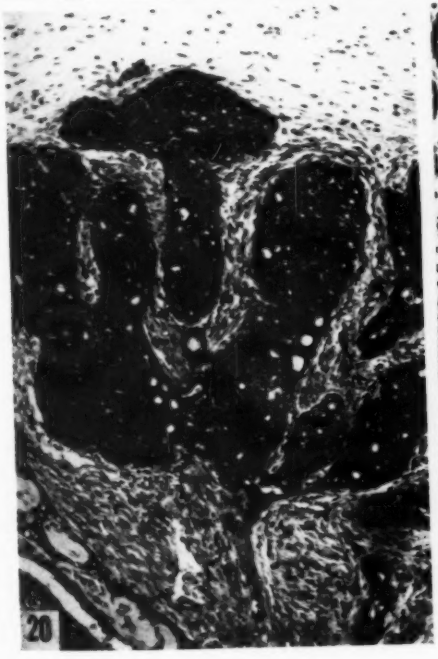
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PLATE 71

FIGURE 22.—Carcinoma with much diversity of structure. Organoid areas are present, especially in the *upper central* portion. (Strain BR4 blue F_4.) × 15

FIGURE 23.—Higher magnification of the *upper central* portion of figure 22 showing irregular tubular growth with branching of tubules and conspicuous end-bulbs at the edge of the tumor at the *upper* border of the photograph. × 60

FIGURE 24.—Focal progression to fissured carcinoma in a plaque. (Strain BR4 pink F_4.) × 15

FIGURE 25.—Giant plaque with a single medulla and a convoluted cortex resembling fissured carcinoma. (Strain BR6 F_3.) × 15

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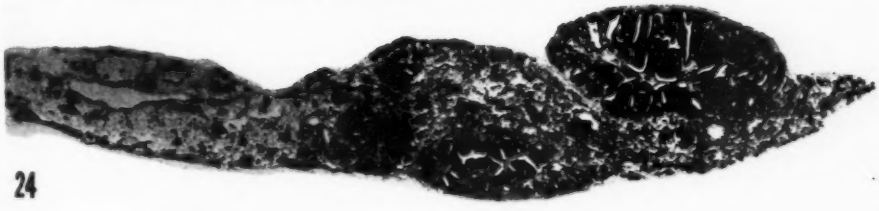
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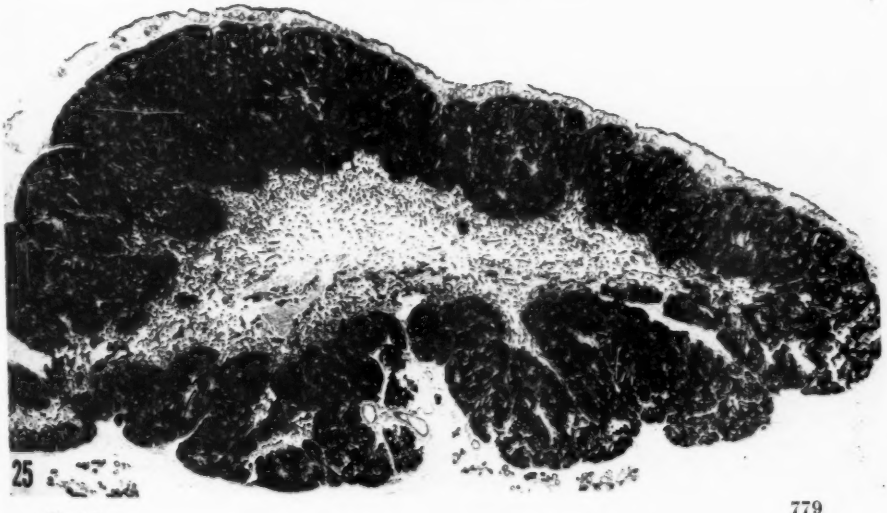
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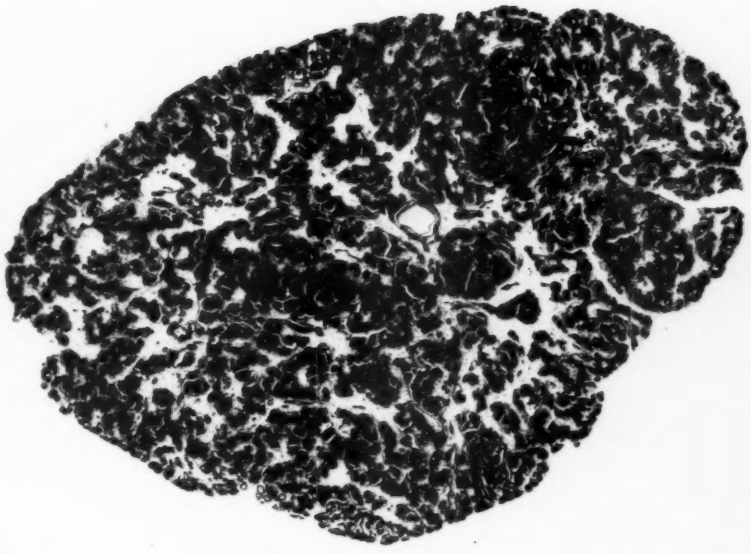
PLATE 72

FIGURE 26.—Tumor composed of separated units of fissured growth. (Strain BR6 F_2.) × 15

FIGURE 27.—Edge of the same tumor showing organoid structure. × 60



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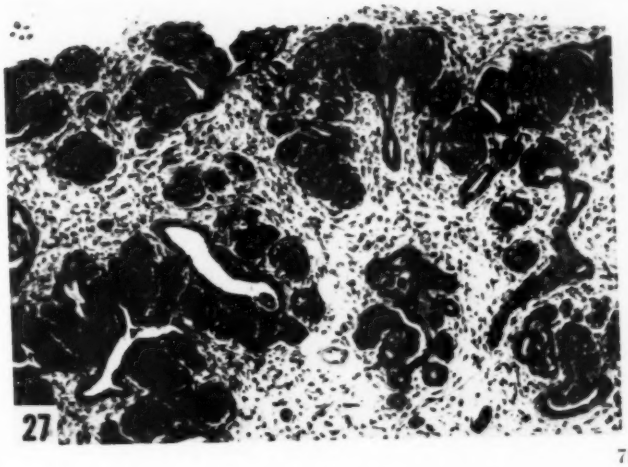

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The Histologic Analysis of Mammary Tumors of Mice. IV. Secretion [1,2]

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In accordance with principles outlined earlier (*1*), this paper deals with the manifestation, at different stages of mammary neoplasia in mice, of 1 conspicuous unit character, namely, secretion and, in particular, with milky secretion—the distinctive activity and *raison d'être* of the parent tissue.

Bonser (*2*) first clearly described milky secretion in mammary tumors of mice and gave illustrations of secreting tumors from lactating female and estrogenized male mice. Subsequent confirmatory observations lack information about the hormonal circumstances (*3*). It is well known that spontaneous and transplanted mammary fibroadenomas in rats secrete in parallel with the normal breast during pregnancy and lactation and, in consequence, the structure changes to an adenomatous or cystadenomatous type. In women some benign mammary tumors secrete but carcinomas do not (*4*). Dawson (*4*) describes secretion, probably milky, in a "mixed" tumor from a lactating dog. Eisen's (*5*) description of milky secretion in response to estrogenic stimulation in a transplantable mammary carcinoma in rats seems to be the only account of an experimental study of secretion in mammary carcinoma.

Material and Methods

The material and methods used in this investigation were described previously (*1*). The periodic acid-Schiff (PAS) method was used more frequently for the study of secreted materials. It is well to recall that most of the tumor-bearing mice were subjected to forced breeding, which often continued up to the time of examination.

Secretion in Normal Mouse Breast

The detailed description by Dawson (*4*) of secretion in the human breast does not necessarily apply at all points to secretion in rodents but a broad

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[2] This investigation has been supported by grants to the Royal Cancer Hospital and the Chester Beatty Research Institute from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

similarity in all the species emerges from the observations of Turner and Gomez (*6*), Cole (*7*), Wieser (*8*), and Fekete (*9*) on mice, and those of Roberts (*10*), Maeder (*11*), and Curtiss (*12*) on rats. In mice, a single layer of cells lines the secreting acini. Droplets of fat, at first small, appear in the basal portion of the cell, increase in size, move toward the free border pushing the nucleus to the base of the cell, and discharge through a break in the cell wall into the lumen of the acinus. Duct epithelium secretes likewise but the droplets are smaller.

There seems to be no published account of secretion in mice subjected to forced breeding. Incidental observations on normal breast made in the course of the present study correspond in general with the earlier accounts of secretion. As a result of secretion the acini distend and their epithelial lining flattens. The secreted material, as described by Dawson and by Maeder, consists of a granular eosinophilic coagulum enclosing droplets of fat. The eosinophilic material stains positively by the PAS method and is present in varied amounts; it seems to be more abundant in ducts than in acini and it is conspicuous in involuting glands. As mentioned by Apolant (*13*) and more specifically by Wieser (*8*) a homogeneous eosinophilic material that is PAS-positive is present in the lumina of resting glands and apparently increases during pregnancy before secretion of fat begins. Inspissated secretion and milk cysts often persist for a long time after weaning.

Secretion in Mammary Neoplasia

Secretion in mammary tumors sometimes broadly resembles that in the normal breast (*13*), but differences are to be expected and, for closer analysis, different types of secretion need to be distinguished.

Milky secretion, with which this paper is primarily concerned, nearly resembles normal secretion. Fat droplets appear within the cytoplasm of tumor cells, increase in size, move toward the free margin, and thence are discharged, leaving the cell temporarily with a ragged or concave border. The secreted material forms a vacuolated mass of fat droplets and mucinous, PAS-positive material in greatly varied proportions (fig. 12).

Mucinous secretion is commonplace in mammary tumors and occurs alone as well as in conjunction with fat secretion. The secreted material is eosinophilic and PAS-positive. The mode of secretion is not evident. Some types of secretion, for example *watery secretion*, are not distinguishable histologically. Many spaces which in histologic sections are empty presumably contained watery fluid during life. Dawson (*4*) discusses, with references to the earlier literature, the possible role of *transudation* in the progressive accumulation of fluid when continued secretion is not a likely explanation. Edematous changes and cyst formation are extremely common in mammary tumors of mice.

A process, of which traces have been seen in normal breast, is evident in many tumors but is conspicuous only in small areas. Tongues of cytoplasm protrude from the free borders of tumor cells lining tubules or

cysts (figs. 1 and 2). Some of the protrusions are much elongated and narrowed at their attachment to the cell. The constriction at the base seems to increase until the protrusions are pinched off to form spheres which rest on the epithelium or scatter in the spaces (fig. 3). The protrusions and the spheres stain similarly but not distinctively; in general they stain like the cell cytoplasm. This process of *budding* is akin to the previously described phenomenon of "decapitation," whereby a portion of the epithelial cell with its included secretion is shed into the gland lumen. Dawson (*4*) and others are skeptical about this type of secretion in the human breast. Cole (*7*) found no evidence of "decapitation" in mice nor did Maeder (*11*) in rats, but Roberts (*10*) mentions it as a rare event in rats and Wieser (*8*) thinks that decapitation ("abgestossen Zellkuppen") is a possible source of eosinophilic secretion in normal mouse breast. It is not certain that the *budding* now described is always a true "decapitation" of cells but it seems to represent a distinct form of liberation of material from tumor cells.

The general principle that secretion of milk is inhibited by accumulation of secreted material is applicable to tumors. There is no egress from tumors. Consequently, either the secretion is self-limiting and stops when distension has advanced to a stage of cystadenoma or else the containing walls break and, as a result, further secretion becomes possible and large milk cysts or a distinctive type of secreting papillary intracystic growth develop. Self-limiting secretion is most conspicuous in early stages of neoplasia whereas in carcinoma the alternative process is more evident.

Secretion in Early Stages of Mammary Neoplasia

Some of the hyperplastic nodules described by Haaland (*14*) secreted in parallel with normal breast but others were inactive while normal breast was secreting. Fekete (*9*) also found both secreting and nonsecreting nodules and thought that those nodules that failed to secrete during late pregnancy and lactation were more liable to progress to carcinoma. Other observers described what appears to be the more common finding—secreting nodules in breasts which elsewhere were inactive or involuted (*2*, *15–17*).

The plaques, which are the most characteristic expressions of early neoplasia in the present material (*18*), behave as a rule like some of the nodules described by Haaland and by Fekete and do not secrete during late pregnancy. Eosinophilic material often fills the tubules in partially regressed plaques but milky secretion is exceptional. Judging from the few examples available, secretion begins in the centrally placed tubules, which become distended and, when pronounced, extends outward until most of the tubules contain secreted material (fig. 4). Sometimes an incomplete rim of end-bulbs alone remains free from secretion. The tubules are variously but not maximally distended or mutually compressed (fig. 4). Their epithelium is usually cuboidal and shows varied degrees of secretory vacuolation. At this stage the growths correspond closely with Apolant's (*13*) "cysta-

denoma simplex"; after further secretion they come to match Apolant's "papillary cystadenoma with scanty stroma." The different stages merge with each other, and secretion may advance to varied degrees in different tumors in the same mouse. The separation of "cystadenoma" as a distinct histologic type is arbitrary, but the term is convenient and is used here for tumors with the distinctive structure attained when secretion reaches its maximum (figs. 5 and 6). The fully developed cystadenomas, of which my material contains 7 examples in 5 mice, resemble ordinary plaques in shape and size, their longest diameters ranging from 0.2 to 1.0 cm. They are smoothly outlined but not encapsulated; microscopically, 1 otherwise typical cystadenoma appears invasive (fig. 5). The tumors are composed of widely distended tubules, tightly packed and mutually compressed, with minimal intervening stroma (fig. 6). Most of the tubules are lined by a single layer of flattened inactive epithelium; a minority have cuboidal epithelium with occasional droplets of secretion within the cells. The secreted material contains droplets and varied, usually large, amounts of eosinophilic, PAS-positive material. In spite of the distension and distortion of tubules by accumulated secretion, radially disposed branching tubules are discernible in 3 of the most characteristic cystadenomas (fig. 6).

Of the 5 mice with typical cystadenomas 2 had been nursing litters for 3 and 10 days, respectively, and 3 bore full-term or post-mature fetuses. Cystadenoma reaches full development at the end of pregnancy or during lactation. Bonser's (*2*) illustration of a similar tumor from a male mouse estrogenized for 65 weeks indicates that artificially administered hormones can substitute for the normal lactogenic stimuli.

The ultimate fate of cystadenoma is uncertain because no example has been recognized during life. No cystadenoma in this series is large and none derives from a mouse which was not in late pregnancy or lactating. In the absence of lactogenic stimuli cystadenoma probably regresses quickly or undergoes progression to carcinoma. Tumors from 3 mice which had littered from 3 to 10 days earlier and had not nursed show probable regressing phases of cystadenoma. Atrophic or nonsecreting epithelium lines variously distended tubules which are more widely separated by stroma than in typical cystadenoma and interspersed with undistended tubules. The distended tubules contain inspissated material, often with concentric markings (fig. 8). These tumors fit into another of Apolant's subdivisions of cystadenoma (*see* his fig. 6: "Papillăres Cystadenome mit relativ viel Stroma und geschichteten Inhaltmassen"). Two other tumors are possibly regressed cystadenomas and the counterparts of the sclerotic plaques described previously (*18*). They have a honeycombed structure (fig. 7) with spaces lined by cuboidal or, more usually, by flattened epithelial cells; they came from mice which had not been pregnant for 2 months.

The inference from these observations is that cystadenoma is the result of self-limiting secretion in response to hormonal stimulation at an early stage of neoplasia. The secretion is dependent upon a stimulus supplied by late pregnancy, lactation, or artificially administered hormones and

upon the capacity of the tumor to respond to the stimulus. The responsiveness is exceptional at this early stage of neoplasia, which ordinarily, in this material, takes the form of a plaque composed of nonsecreting, radially disposed branching tubules. Most plaques grow during pregnancy and regress immediately after parturition whether or not the mouse nurses its litter, and they do not secrete at any time. Cystadenomas, by contrast, grow during pregnancy, persist during lactation, and secrete in parallel with the normal breast. Physiologically, cystadenoma is the smallest recognizable neoplastic deviation from normal breast. The histologic structure is the consequence of the near-normal responsiveness to hormones, and the varied degrees of secretion reflect different degrees of responsiveness. The various subdivisions of cystadenoma distinguished by Apolant are different stages of 1 process and correspond with different stages of reproduction.

Milky Secretion in Primary Carcinoma

Early secretion is manifested by widespread vacuolation of tumor cells without great distension of tubules or substantial modification of the tumor's architecture (fig. 9). Occasionally it is questionable whether the accumulation of fat should be ascribed to secretion or to fatty degeneration. At a later stage, self-limiting secretion may lead to the development of adenomatous or cystadenomatous areas, but more commonly tubules rupture and form irregular cystic spaces with ragged walls lined by epithelial cells in irregular arrangement and often in many layers (figs. 10 and 11). The tumor cells contain small or large vacuoles which discharge into the lumen leaving the free border of the cell concave or ragged (fig. 12). The secreted material is much vacuolated and contains as a rule only small amounts of acidophilic mucinous material. The secreting tumors derive mainly from mice killed during late pregnancy. Tumors from mice not in late pregnancy but with a history of recent pregnancy often have a similar architecture, but signs of active secretion in tumor cells are lacking and the cystic spaces may contain inspissated material. The histologic appearance is consistent with active secretion during the antecedent pregnancy. Budding is frequently but never extensively present and its correlation with reproductive activity is dubious; it is more conspicuous as a rule after milky secretion has stopped.

The distribution of secreting tissue is always patchy; no tumor is composed wholly of secreting tissue and in many tumors the secreting tissue is small in amount and inconspicuous. It is conceivable that secretory activity is cyclical in tumors as it is in the normal breast in women (*4*) and that some portions are always in the resting stage while others are secreting. Secreting and nonsecreting epitheliums are sometimes seen side by side and in continuity with each other. The nonsecreting epithelium is the more regular and provides a smooth lining for the cystic spaces, the resulting structure resembling that of nonsecreting papillary intracystic carcinoma (figs. 13 and 14), as described by Cloudman (*19*)

and by Dunn (*3*). It is not certain that secreting tumors always revert to ordinary papillary intracystic carcinoma when secretion stops or that papillary intracystic carcinoma is always, or usually, the result of antecedent secretion. These possibilities need further investigation.

In general, observations indicate that milky secretion occurs during late pregnancy and lactation and stops after weaning. Some apparent exceptions to the rule are accountable to incomplete recording of pregnancies for which there is good evidence in the form of recurrence of a responsive tumor or secretion in normal breast. Possibly real exceptions occur, but in my material they are rare. A survey of 125 secreting tumors disclosed 1 or possibly 2 examples of secretion in mice that almost certainly were not pregnant but in these ovarian abnormality or other sources of excessive hormone production were not excluded.

The frequency of milky secretion is difficult to assess. The histologic notes made during this investigation record probable milky secretion, active or recent, in 175 tumors from 131 mice out of a gross total of 870 tumors from 710 mice examined. The apparent incidence of roughly 20 percent is probably an underestimate. Owing to its patchy distribution, milky secretion easily escapes notice in random sections as used in this investigation and unless deliberately sought with a degree of care that was not applied consistently to all the tumors. The 131 mice with 175 secreting tumors had, in addition, 105 tumors in which secretion was not seen or recorded. Allowing for errors in observation and recording, it seems fairly sure that different tumors in a single mouse react differently to the same lactogenic stimulus. The occurrence and the degree of secretion depend on the responsiveness of individual tumors to the appropriate hormones.

Milky Secretion in Transplanted Tumors

An earlier paper (*20*) recorded milky secretion visible to the naked eye in some transplanted mammary tumors and especially in the 4th, 5th, and 6th transplant generations of line CRI. In early passages CRI grew well in female or estrogenized male hosts but tardily or not all in normal males. After the 6th or 7th passages CRI grew equally well in male and female hosts. The conspicuous milkiness was in tumors from females in late pregnancy and from estrogenized males. It disappeared when the tumor acquired the capacity to grow in males without the aid of estrogen and did not reappear in a subline maintained for 37 generations.

Histologic material is available from 44 tumors of the generations in which gross milkiness was seen. It confirms the naked-eye observations and establishes the correlation between milky secretion and late pregnancy or administration of estrogen. Milky secretion is present in tumors from 8 out of 15 female mice; 7 of the 8 mice with secreting tumors were in late pregnancy and the reproductive history of the 8th is not known. In addition, 3 mice in early or mid-pregnancy had tumors with a histologic appearance consistent with secretion during an earlier pregnancy. Milky secretion is evident in 18 out of 19 tumors from estrogenized male mice,

but is only slight or doubtful in 1 mouse in which the estrogen pellet was not found *post mortem*, although atrophy of the male accessory sex organs gave evidence of prior estrogenic stimulation. The 1 nonsecreting tumor came from a mouse in which no pellet was discovered *post mortem* nor any sign of estrogenic action on the male sex organs. Removal of estrogen pellets from male mice after implants had become established retarded growth but did not lead to regression; none of the tumors from these males was milky to the naked eye and none of the 3 available for histologic study was milky. No milky secretion is present in 3 tumors from ovariectomized females or in 4 tumors developing late in normal males. These results, summarized in table 1, show that milky secretion occurred only in response to the stimulus of late pregnancy in females or estrogenic stimulation in males.

TABLE 1.—*Histologically confirmed milky secretion in transplanted CRI tumors*

Host mouse	Total tumors	Tumors with milky secretion	Remarks
Normal ♀	15	8	7 In late pregnancy. 1 Reproductive history unknown. (Signs of secretion during antecedent pregnancy in 3 others.)
Ovariectomized ♀	3	0	
Estrogenized ♂	19	18	No secretion in 1; estrogen pellet not found *P.M.* and no evidence of action on ♂ sex organs. Slight secretion in 1; estrogen pellet not found *P.M.* but evidence of action on ♂ sex organs present.
Estrogenized ♂ after withdrawal of estrogen.	3	0	
Normal ♂	4	0	

The secreting transplanted tumors are of the distinctive papillary intracystic type already described (fig. 15). They differ from secreting primary tumors mainly in the greater amount of secreting tissue and the more conspicuous milk cysts. Active secretion in tumor epithelium is often slight, especially in the tumors long subjected to estrogenic stimulation in male mice; presumably secretion is inhibited by the accumulated products or the epithelium is exhausted by previous activity. The accumulations of milky fluid are greatest in estrogenized male mice. The non-milky tumors are of predominantly microtubular or irregular tubular structure and similar nonsecreting tumor tissue is present to a greater or lesser extent in most sections of milky tumors. It is unfortunate that only 3 tumors are available for studying the effect of estrogen withdrawal. These tumors have the same histologic appearance as the non-milky tumors already mentioned, although it is almost certain that

milky secretion was present during the earlier period of estrogenic action (fig. 16). The inference, needing further investigation, is that the distinctive secreting type of papillary intracystic growth may revert to microtubular or irregular tubular structure after withdrawal of lactogenic stimulation so that no histologic indication of the earlier secretion persists. Alternatively, as the observations on primary tumors suggest, some of the secreting tumor persists as ordinary papillary intracystic carcinoma but is submerged by further growth of tumor of nonsecreting type. Areas of nonsecreting papillary intracystic structure are rare in CRI at all stages of transplantation.

Non-Milky Secretion in Mammary Carcinoma

Less attention was given to non-milky secretion and the observations add little to previous accounts. Apolant (*13*) states that the acini of *carcinoma simplex* (here called *microtubular carcinoma*) usually contain, as do the acini of normal breast, an acidophilic, colloidlike material which either fills the lumen or is irregularly retracted from the walls. Dunn (*21*) illustrates this kind of secretion in a tumor with ". . . lumina dilated and containing a clear fluid. . . ." and supports Apolant's remark about the resemblance to colloid goiter. Bonser (*2*) also records cystic tubules filled with eosinophilic secretion in irregular tubular carcinoma. The present observations confirm the frequency of eosinophilic, PAS-positive secretion, presumably mucinous, and indicate that it is independent of reproductive activity.

The non-milky papillary intracystic carcinoma to which reference has been made is characterized by papillae, covered with a regular epithelium, projecting into smooth-walled cysts (*19,21*). According to Bonser (*2*), the cysts contain albuminous fluid or blood. This type of structure is common in my material. It is independent of reproductive activity. Some cysts contain PAS-positive material but many are apparently empty. Apolant describes the modification of acinar carcinoma into fissured ("spältenbildende") carcinoma and thence to papillary carcinoma and says that only few of the spaces in fissured carcinoma contain secretion masses. His figure 17 illustrating papillary carcinoma is consistent with antecedent milky secretion. The present observations suggest the possibility, needing further study, that papillary intracystic growth is sometimes the result of antecedent milky secretion, although transplantation experiments showing that papillary intracystic growth is a stable type persisting through many successive generations (*22*) discount prior milky secretion as a sole determinant of the structure.

Discussion

Milky secretion depends as a rule on a lactogenic stimulus provided by late pregnancy, lactation, or artificially supplied hormones and, no less importantly, on the responsiveness of individual tumors to the stimulus. All tumors, even apparently similar tumors in a single mouse, do not

respond to the same degree and some do not respond at all. My material provides no decisive evidence whether or not milky secretion can occur without extrinsic hormonal stimulation and has little direct bearing on Pullinger's (*23*) proposition that secretion can proceed without an extrinsic stimulus. Secretion that is not milky certainly occurs in nonbreeding mice. Judging from the present observations, milky secretion sufficient to produce gross milkiness is always referable to extrinsic hormonal stimulation and so also, with only little less probability, is milky secretion great enough to modify the histologic structure substantially. The present material does not allow a quantitative study of the degree of responsiveness or the intensity of stimulus needed to evoke a secretory response. The response is not an all-or-none effect. The degree of responsiveness and, inversely proportional to it, the required intensity of stimulus probably vary from one tumor to another. Prolonged intense estrogenic stimulation in male mice seems to elicit the maximum response. In Eisen's (*5*) experiments with a transplantable rat carcinoma, milky secretion was dependent upon estrogenic stimulation far above physiologic levels. The relative frequency of milky secretion in primary tumors as now reported is probably attributable to a strain characteristic of the mice whose tumors are unusually responsive to lactogenic stimuli of physiologic intensity. Milky secretion is probably rare in random-bred mice and in many inbred strains unless the hormonal stimulation is intense. The omission of plain reference in the descriptions and illustrations of Apolant (*13*) or of Murray (*24*), Haaland (*14*), or Bashford (*22*) almost certainly means that milky secretion was rare, inconspicuous, or absent in their material. Dunn makes no mention of milky secretion in her earlier review (*21*) but describes it in a later one (*3*). It seems, therefore, that conspicuous milky secretion is an outcome of selective inbreeding and artificial hormonal stimulation.

The histologic studies show that certain distinctive types of structure result from milky secretion, which, in turn, is the consequence of a particular kind of responsiveness to hormones. Cystadenoma, an almost "pure" histologic type, is the result of self-limiting secretion in response to lactogenic stimuli operating on an early stage of mammary neoplasia. In carcinoma, the walls of tubules and cysts are presumably less robust and commonly they rupture under the pressure of secreted material, which results in distinctive papillary intracystic structure. There is no evidence that the capacity for milky secretion is correlated with a particular type of initial histologic structure. The histologic structure of nonsecreting portions of milky tumors or of the transplanted tumors not stimulated to secretion is not remarkable. Histologic examination discloses the consequences of secretory response to hormonal stimulation but not the capacity to respond; secretory response is not predictable from histologic examination of the unstimulated tumor.

The profound effects of active or recent secretion upon histologic structure are evident, but it is not clear whether the effects are transitory or lasting. Some observations on primary tumors suggest that secreting

tumors become commonplace nonsecreting papillary intracystic carcinomas when the hormonal stimulus ceases to act, whereas the transplanted tumors seem to revert to a tubular type when hormone is withdrawn. The late consequences of milky secretion need much further investigation. At present, it is clear that satisfactory histologic analysis of mammary tumors demands a knowledge of current or recent hormonal stimulation and possibly of remote hormonal stimulation as well. The hormones that modify the histologic structure of a definitive tumor are not necessarily the same as those concerned in its initiation, although there is evidence that the hormonal circumstances at initiation may determine histologic type (*25*).

Tumors respond variously to the same or different external stimuli. Milky secretion depends on a particular kind of responsiveness. All or most plaques, which are the characteristic manifestations of early neoplasia in this material, are responsive to hormones and are dependent on pregnancy hormones for growth, but only a small minority have the the special form of responsiveness which entails milky secretion and development of cystadenoma. The transplanted CRI tumors show a linkage between response by secretion and dependence upon estrogenic hormones for the growth of implants, the secretory response and the dependence being lost simultaneously by progression in the course of serial transplantation; yet the linkage is not obligatory, since other strains do not secrete, although, like CRI, they are dependent on hormones for growth. The responsiveness evidenced by secretion is to a large extent an independent character. It is not dependent upon a particular type of histologic structure or upon a special kind of biological behavior. The observations on primary and transplanted carcinomas reinforce earlier conclusions to the effect that responsiveness or even dependence can exist along with all the orthodox signs of malignant growth. Like other characters responsiveness to lactogenic stimuli is liable to independent progression and may manifest itself during only a portion of the life history of a tumor. This particular type of responsiveness is of especial interest because the response is visually conspicuous and attributable, with more confidence than response by growth, to a direct action of the hormonal stimulus upon the tumor cells.

Summary

Milky secretion occurs in malignant and transplanted, as well as benign, mammary tumors of mice. It is usually the manifestation of a particular type of responsiveness to the hormonal stimulation provided by pregnancy, lactation, or artifically administered estrogens. Cystadenoma is the consequence of self-limiting secretion at an early stage of neoplasia. In primary and transplanted carcinoma the walls of spaces distended with milky secretion often rupture under pressure, resulting in the formation of milk cysts and a distinctive type of secreting papillary intracystic growth. The histologic structure is the consequence of responsiveness but histologic examination does not allow the recognition

of responsiveness in the absence of the appropriate stimulus to secretion. The responsiveness is present in varied degrees in some mammary tumors of mice but not in all. The analysis of the histologic structure of mammary tumors of mice calls for a knowledge of both recent and remote hormonal stimulation and a recognition of the histologic consequences of responsiveness thereto.

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PLATE 73

FIGURE 1.—"Budding" from the lining epithelial cells of a cystic tumor from a non-pregnant mouse. (Strain BR6 F_{24}.) × 320

FIGURES 2 AND 3.—Areas of figure 1 showing budding and detached spheres in the spaces. × 720

FIGURE 4.—Plaque (0.6 × 0.6 × 0.4 cm.) with milky secretion especially in medullary tubules to the *right* of the field. The mouse died with 2 post-mature fetuses *in utero*. (Strain BR6 F_3.) × 60

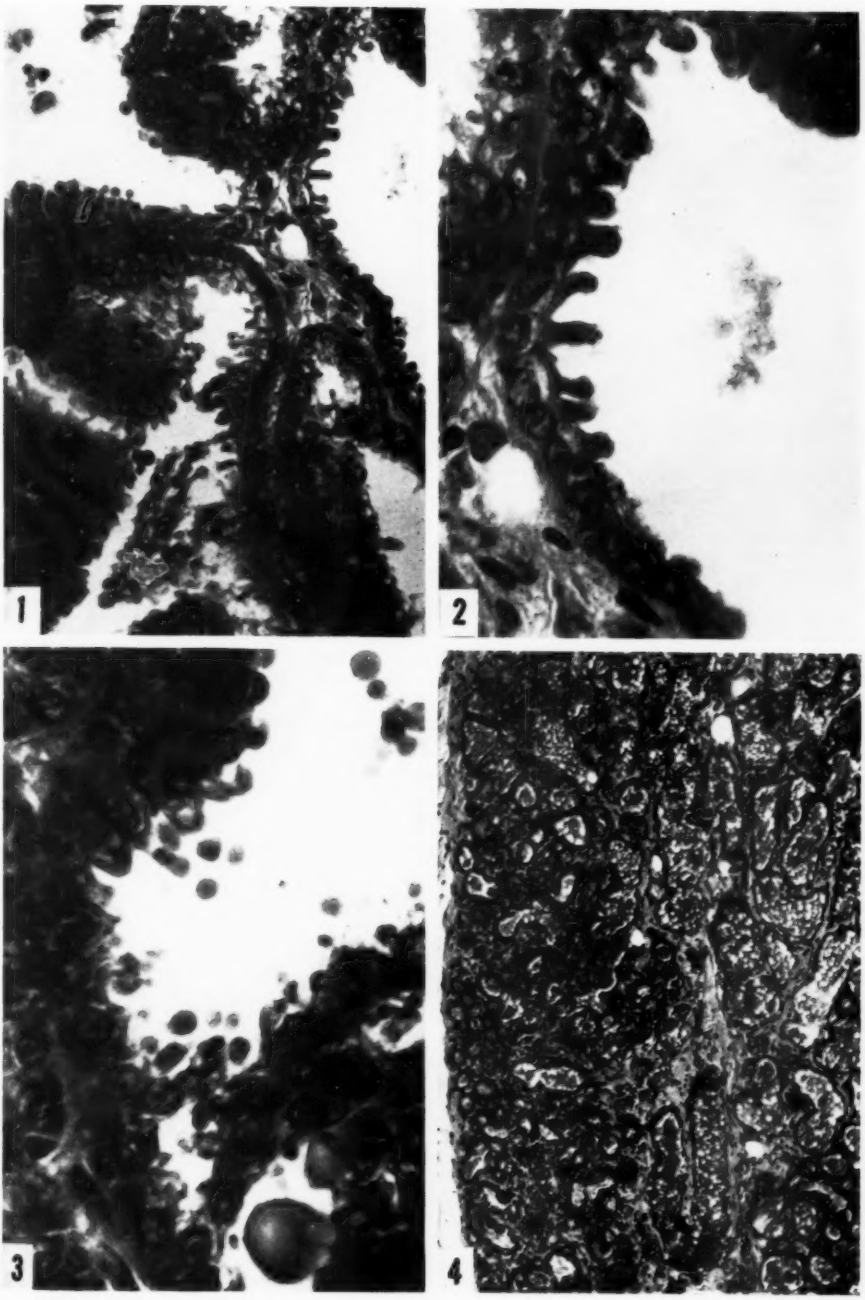

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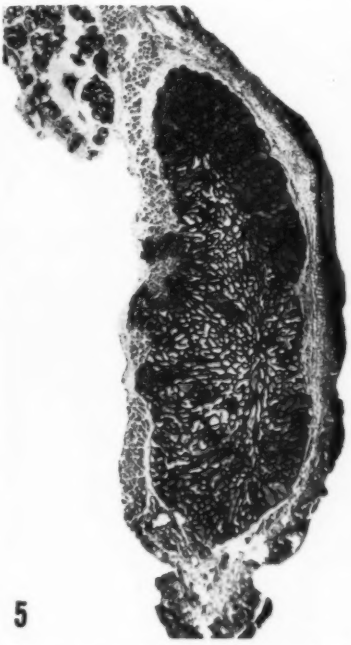
PLATE 74

FIGURE 5.—Cystadenoma with invasion of muscle at the *left* edge. The mouse had been nursing for 3 days. (Strain RB F_1.) × 15

FIGURE 6.—Same tumor as figure 5 showing the radial arrangement of branching tubules distended with vacuolated secretion. × 60

FIGURE 7.—Possible regressed cystadenoma from a mouse which had not been pregnant for 2 months. (Strain BA F_1.) × 60

FIGURE 8.—Probable regressed cystadenoma. Inspissated secretion with concentric markings is present in spaces lined by flattened, inert epithelium. The mouse had littered 10 days previously. (Strain BR6 F_3.) × 60



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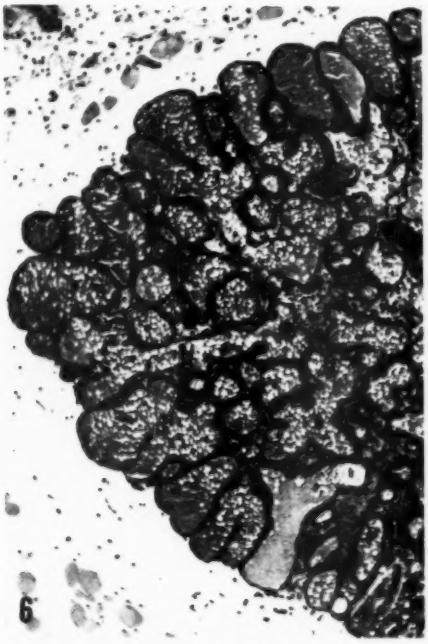

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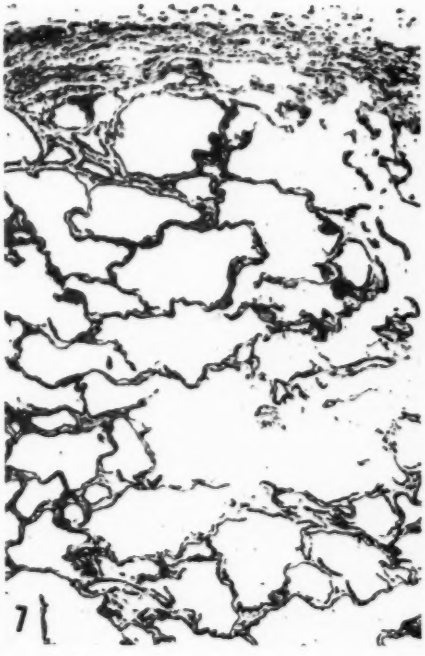

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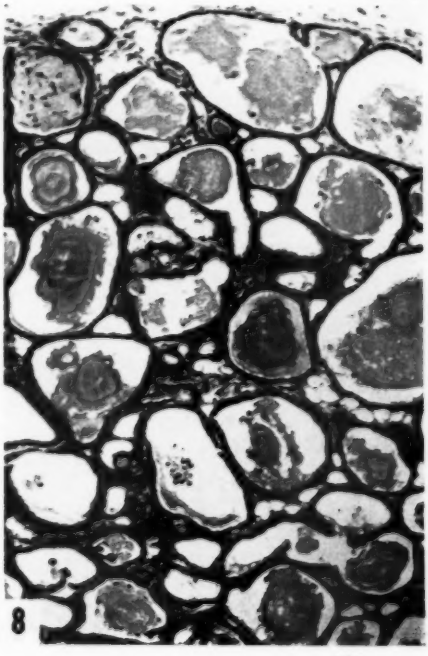

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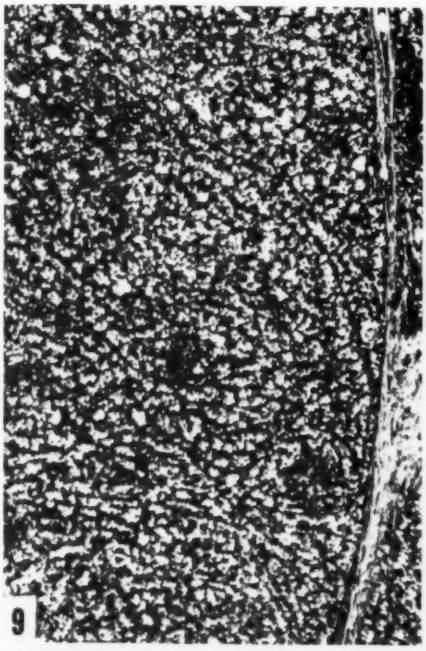
PLATE 75

FIGURE 9.—Diffuse secretory vacuolation in a mammary carcinoma from a mouse in late pregnancy. (Strain BR4 blue F_2.) × 60

FIGURE 10.—Secreting tumor from a mouse in late pregnancy showing breakdown of tubule walls and formation of cysts. (Strain BR4 pink F_3.) × 60

FIGURE 11.—Secreting papillary intracystic tumor from a mouse in late pregnancy. (Strain BR6 F_3.) × 60

FIGURE 12.—An area of figure 11 under higher magnification showing secretion vacuoles in tumor cells and vacuolated secretion in the spaces. × 160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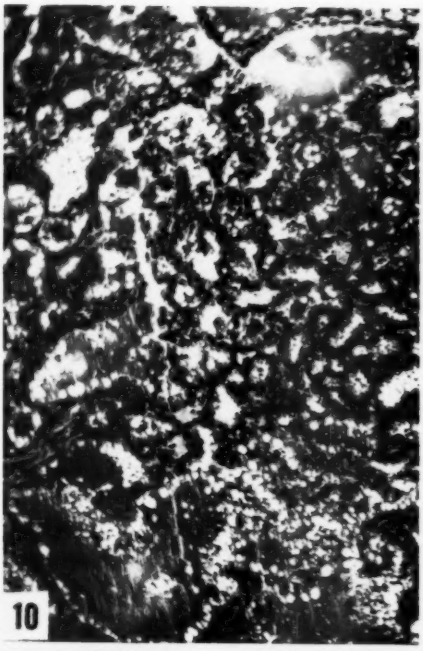
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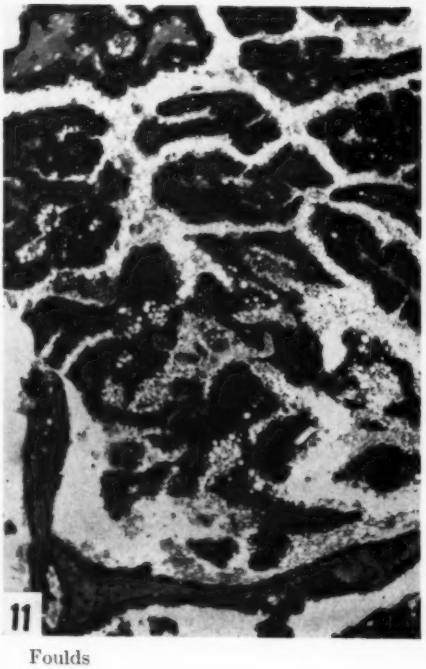
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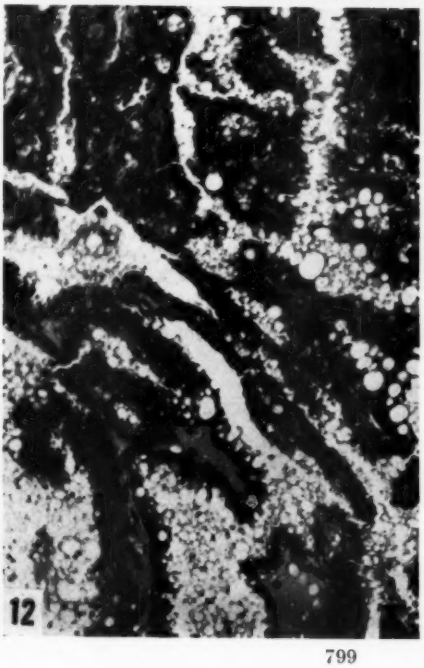
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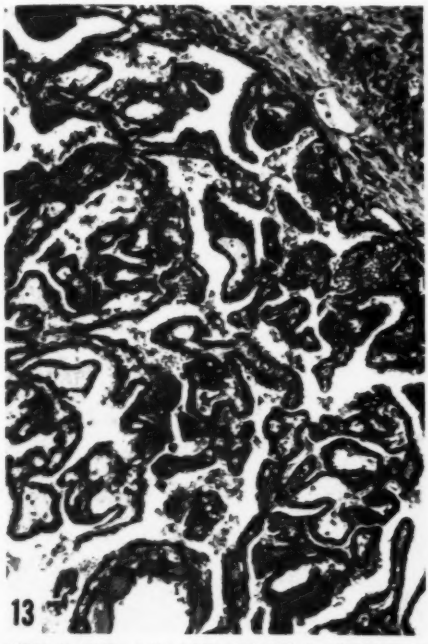
PLATE 76

FIGURE 13.—Tumor from a nursing mouse. There is slight active secretion but most of the tumor resembles nonsecreting papillary cystadenocarcinoma. (Strain BR6 F_{11}.) × 60

FIGURE 14. Tumor from a mouse which littered 4 days previously. Spaces are lined by single layer of nonsecreting epithelium but remains of secretion during the antecedent pregnancy are present at the *right* and *lower* borders. (Strain RB F_1.) × 60

FIGURE 15.—Tumor of 5th transplant generation of CRI growing in a male mouse which carried a stilbestrol-cholesterol pellet up to death. × 60

FIGURE 16.—A tumor of the same series of CRI in a male mouse from which the stilbestrol pellet had been removed 12 weeks before death. × 60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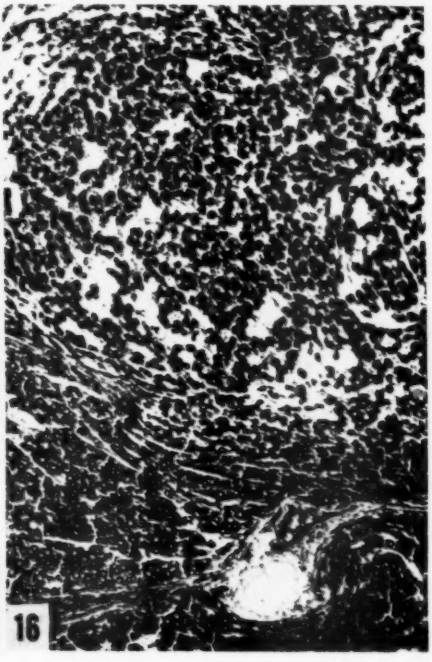
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ANNOUNCEMENT

International Symposium on Mammary Cancer

An international symposium on the development of mammary cancer will be held at the Division of Cancer Research, University of Perugia, Italy, July 24 to 29, 1957. The Symposium will be divided into two parts: One will deal with mammary cancer in animals; the other will be devoted to mammary cancer in human beings.

Papers for presentation at the meeting will be admitted only by invitation, but attendance is open to all interested workers in the field.

Further details will be announced later.

For information write to: Professor L. Severi, Director, Division of Cancer Research, P. O. B. 167, Perugia, Italy.

ERRATA

Volume 17, Number 4, October 1956
Journal of the National Cancer Institute

Page 535: In the paper entitled "The Effect of Subcutaneous Inoculation of 4-*o*-Tolylazo-*o*-Toluidine in Strain HR Mice," by Deringer, in table 1, last column, *for* "Percentage of tumor" *read* "Tumor incidence (*percent*)."

Page 454: In the paper entitled "Observations on a Leukemic Cell Variant in Mice," by Humphreys *et al.*, in the schema the *arrow* pointing from *Tumor* to *Infectious agent* should be reversed, to point toward *Tumor*. The *horizontal broken lines* should each have a *half arrow* at opposite ends—one from *Tumor* to *Host* and one from *Host* to *Tumor*.

The Journal of the National Cancer Institute is published monthly by the National Cancer Institute of the National Institutes of Health, Bethesda, Md. Twelve issues are published each year in two volumes, beginning with number 1 in July and January. The Journal publishes original observations and investigations in laboratory and clinical research in cancer. Manuscripts are considered for publication with the understanding that they have not been published previously.

Publication is not limited to members of the National Cancer Institute or to those whose work is supported by Government funds. Manuscripts should be submitted to the Journal of the National Cancer Institute, National Cancer Institute, Bethesda 14, Md.

Suggestions to Authors

1. Submit an original and two carbon copies of the manuscript. Follow the style of a recent copy of the Journal, especially for illustrations, references, and tables. The latest edition of Webster's International Dictionary is followed in matters of spelling, capitalization, punctuation, compounds, and hyphenation. Current medical dictionaries and Chemical Abstracts are usually followed for medical and chemical terms. Type material double spaced.

2. In the by-line, include the name of the institution with which each author is connected. Official title or academic degree is optional.

3. Begin each page with a new paragraph; *i.e.*, each page should contain one or more complete paragraphs, and no paragraph should carry over from one page to the next unless it is longer than a full page. Do not begin a new paragraph on a page carrying a run-over paragraph. This is a requirement of the Government Printing Office.

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